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Molecular-size dependence of glycogen enzymatic degradation and its importance for diabetes

Xiaoyin Jiang [a], Peng Zhang [a], Shihan Li [b], Xinle Tan [a,b], Zhenxia Hu [a], Bin Deng [a], Kai Wang [d], Cheng Li [b], Mitchell A. Sullivan [c], Enpeng Li [a,b,*], Robert G. Gilbert [b,*]

[a] School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China
[b] The University of Queensland, Centre for Nutrition and Food Science, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD 4072, Australia
[c] Program in Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON M5G 0A4, Canada
[d] College of Food Science, South China Agricultural University, Guangzhou 510642, China

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ABSTRACT

Glycogen, a hyperbranched glucose polymer, is the blood-sugar reservoir in animals. Liver glycogen comprises small β particles, which can join together as large composite α particles. It had been shown that the binding between β in α particles in the liver of diabetic mice is more fragile than in healthy mice. This could be linked to the loss of blood-sugar control characteristic of diabetes if the rate per monomer unit of the enzymatic degradation to glucose of α particles were significantly slower than that of β particles. This is tested here by examining the *in vitro* time evolution of the molecular size distribution of glycogen from the livers of healthy and diabetic mice and rats, containing distinct components of both α and β particles; this treatment is analogous to the "competitive growth" method used to explore mechanisms in emulsion polymerization. Simulations for the time evolution of the molecular size distribution were also performed. It is found that the degradation rate per monomer unit is indeed faster for the smaller particles, supporting the hypothesis of a causal link between chemical fragility of glycogen from diabetic liver with poor control of blood-sugar release. Comparison between simulations and experiment indicate that α and β particles have significant structural differences.

1. Introduction

Glycogen is a highly branched polymer of glucose that comprises two types of particles: small β particles (~20 nm in diameter), which can form large α particles (up to 300 nm in diameter) which have a cauliflower-like appearance under transmission electron microscopy [1–3]. Glycogen predominantly exists in brain, muscle [4], heart [5] and liver, functioning as a buffer to release or store glucose as appropriate.

We have shown previously that liver glycogen in db/db mice (an animal model of type 2 diabetes) has similar amounts of α particles to healthy liver glycogen. However, the α particles from diabetic mice are much more fragile [6], e.g. to solvents which break hydrogen bonds, than those from healthy mice. They would thus be more readily broken down into β particles [7] in the liver. This phenomenon could be related to the hyperglycemia (high blood glucose, loss of blood-sugar control) that

* Corresponding authors at: School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei 430030, China (E. Li), Centre for Nutrition & Food Sciences, QAAFI, The University of Queensland, Brisbane, Qld 4072, Australia (R.G. Gilbert).
E-mail addresses: en.li@uqconnect.edu.au (E. Li), b.gilbert@uq.edu.au (R.G. Gilbert).

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is characteristic of diabetes if the degradation process of β particles to glucose were to be at a faster rate than that of α particles.

This study investigates the relative rates of the degradation processes of α and β particles from the livers of healthy rats, healthy mice and diabetic db/db mice, by examining the time evolution of glycogen molecular size distribution during *in vitro* enzymatic degradation.

Glycogen phosphorylase, which catalyzes the breakdown of glycogen by cleaving (1 → 4)-α glycosidic bonds [8,9], was used to imitate glycogenolysis *in vitro*. Size-exclusion chromatography (SEC, also termed gel-permeation chromatography, GPC), which separates fully dissolved and dispersed polymer molecules by size (the hydrodynamic radius R_h), was used to examine the molecular size distributions of glycogen at different degradation times [10]. Studying the time evolution of bimodal size distributions to make mechanistic inferences is analogous to the "competitive growth" experiments used to develop mechanistic understanding of growth processes in emulsion polymerization [11].

2. Experimental

2.1. Animals

Male Wistar rats (8 weeks), male C57 mice (12 weeks) and male C57BL/6j-db/db mice (12 weeks) were used. Rats and C57 mice were purchased from Hubei Provincial Food and Drug Safety Center. C57BL/6j db/db mice were purchased from the Model Animal Research Center of Nanjing University. Animals were housed in standard cages, with the temperature controlled at 22 ± 1 °C. A 12 h dark/light cycle was used, with lights on at 7 a.m. and off at 7 p.m. Animals had *ad libitum* access to standard chow (6% kcal from fat, 14.3 MJ kg^{-1}, Hubei Provincial Center for Disease Control and Prevention) and water. Animals were anaesthetized with sodium pentobarbitone (150 mg kg^{-1}, i.p.), with their livers rapidly excised and snap-frozen in liquid nitrogen. Samples were stored at −80 °C. All animal experiments were approved by the Huazhong University of Science and Technology Tongji Medical College Animal Care and Ethics Committee.

2.2. Glycogen extraction and purification

Glycogen extraction was carried out following our published method with some modifications [12]. Liver glycogen was homogenized, and a stepwise sucrose gradient (37.5% and 75% in deionized water) method was used to remove non-glycogen components. Glycogen was further purified by ethanol precipitation, and then dissolved in 1 mL of deionized water and lyophilized (VirTis BTP-9EL freeze-dryer).

2.3. Glycogen degradation

Glycogen degradation was analyzed as described elsewhere [12]. This used a buffer solution obtained by mixing 500 mM potassium phosphate (100 mL), 300 mM magnesium chloride (5 mL) and 100 mM EDTA (1 mL) (adjusted to pH 6.8). The digestion buffer was obtained by mixing this solution (1.5 mL) with deionized water (10 mL), NADP (6.5 mM) and 0.1% (w/v) α-D glucose 1,6-disphosphate. 2 mg of liver glycogen was dissolved in 1.2 mL digestion buffer and then incubated at 80 °C with agitation at 350 rpm for 4 h. The following reagents (Sigma) were added in this mixture: glycogen phosphorylase (40 μL, 1 mg mL^{-1}), phosphoglucomutase (24 μL, 10 U mL^{-1}), glucose-6-phosphate dehydrogenase (24 μL, 10 U mL^{-1}) and 5′AMP (2 μL, 100 mM). Samples were collected after 0, 20 and 60 min, and absolute ethanol added (4 times) to terminate the reaction. These times were chosen so as to cover the range from the time the degradative enzyme was added to when the glycogen was almost completely degraded. Digested glycogen was collected after centrifugation at 4000*g* for 10 min, and was re-dissolved in 0.3 mL of deionized water and lyophilized (freeze-dryer; VirTis, BTP-9EL) for SEC analysis.

2.4. Size-chromatography (SEC) of glycogen

The SEC weight distribution of glycogen, $w(\log R_h)$, which gives the distribution of the weight of particles as a function of $\log R_h$, was analyzed using an Agilent 1260 Infinity SEC system (Agilent, Santa Clara, CA, USA) with a refractive index detector (RID, Optilab UT-rEX, Wyatt, Santa Barbara, CA, USA) following a previously reported method [6]. Water containing 50 mM ammonium nitrate/0.02% sodium azide was used as eluent, and separation of molecules by size used a SUPREMA pre-column, 1000 and 10,000 columns (Polymer Standard Service, Mainz, Germany).

3. Simulations

Two approaches were used to simulate the time evolution of the molecular size distribution under enzymatic degradation. Both were based on analogous methods used in emulsion polymerization. The first approach was through the partial differential equation involving a single model describing this evolution, analogous to the full description of the particle size distribution in emulsion polymerization during particle formation and growth [13], and is an extension of that used