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Note

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The Mechanism for Stopping Chain and Total-Molecule Growth in Complex Branched Polymers, Exemplified by Glycogen

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Supporting Information

INTRODUCTION

Complex highly branched polymers have many applications: e.g., dendrimers as drug carriers,[1] hyperbranched synthetic polymers as rheology modifiers,[2] glycogen as the blood-sugar (glucose) reservoir in animals, and starch as both the glucose storage polymer for plants and the largest component of human food energy. The functional properties of these polymers depend on their molecular structure. One structural feature of significance is the molecular weight distribution of individual branches (or chain-length distribution, CLD, in the parlance used for starch). This is controlled by two rates: those of chain growth (propagation) and of chain stoppage. Chain stoppage mechanisms include transfer, abstraction or radical−radical termination for free-radical polymerization, and, for starch and glycogen, by branching enzyme (BE).

For unbranched polymers (with only one chain), chain stoppage also stops the growth of the whole molecule. However, the only "chemical" mechanisms stopping the whole (branched) molecule growing indefinitely are depletion of monomer or of one of the reactants or catalysts for chain growth. There can also be physical events stopping growth of the whole molecule and of an individual chain. A prime example of these is steric hindrance (also termed crowding), for example, in dendrimers[3] and glycogen.[4−6]

This Note examines the chain-stopping mechanism in glycogen; the results should also shed light on the growth-stoppage process for the whole molecule. The inferences from this study are probably relevant to different types of complex branched polymers.

Glycogen is a randomly branched glucose polymer with α-(1 → 4) linear links and α-(1 → 6) branch points; it contains small but significant amounts of protein, so is actually a proteoglucan.[7,8] BE removes from the end of a growing chain a piece of chain of degree of polymerization (DP) X, greater than or equal to a minimum value X_{min}, leaving a chain with DP greater than or equal to another minimum value X_0, then, with the piece that has been removed, forming a new branch on any chain. This results in the formation of two new growing chains (both shorter than the original). Propagation is by glycogen synthase.

Glycogen has several advantages for studying chain stoppage. (1) Each branch point can be quantitatively cleaved enzymatically,[9] enabling the CLD to be accurately determined. (2) The branch points are randomly distributed. (3) The "chemical" events in glycogen biosynthesis are simple:[10] a single propagation enzyme, glycogen synthase, and a single mechanism for branch formation, with a single BE.

MATERIALS AND METHODS

Extraction and preparation of glycogen samples, reagents, debranching and measuring the CLD using fluorophore-assisted capillary electrophoresis (FACE) followed techniques described elsewhere[11−13] (see Supporting Information (SI)). FACE gives the number CLD as a function of X, $N_{de}(X)$, directly and accurately, with baseline resolution between DPs in the range of interest. Liver samples were from mice sacrificed at different times of day, and from excised portions of human livers after 12 h of fasting. Approval for the use of animals was through the Huazhong University of Science and Technology Tongji Medical College Animal Care and Ethics Committee. Informed consent was obtained from each patient for the human samples. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Human Research Committee of Huazhong University of Science and Technology and Wuhan General Hospital of Guangzhou Military Command.

RESULTS AND DISCUSSION

Selected CLD data are presented in Figure 1 on a logarithmic[14,15] Y axis. Data for all samples are in the SI, showing that those in Figure 1 are indeed representative.

Human and mouse glycogen CLDs have similar features, although their distributions vary significantly. There is some variation between CLDs of individual mice, but no apparent dependence on time of sacrifice. Both species clearly show two CLD components, implying two processes for chain growth/stoppage. Multiple CLD components occur with starch (with a very similar molecular structure to that of glycogen), because there are several different isoforms (types) of each enzyme type.[15] However, for glycogen, it is widely believed that there is

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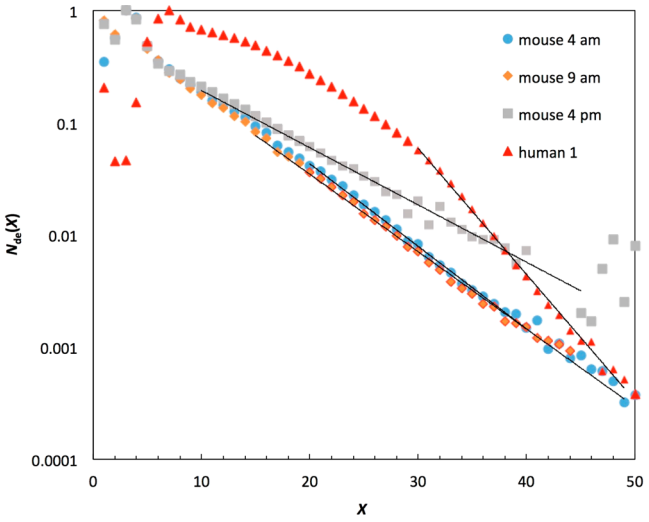

Figure 1. Typical CLDs of liver glycogen from mice sacrificed at three different times, and of human liver glycogen after 12 h of fasting, normalized to the peak maximum. Lines are straight-line fits to the higher DP region.

only one isoform of each of the two biosynthesis enzymes[16,17] (although some results have been interpreted, using indirect evidence, to suggest that there are two synthases[18]). Hence there is some other mechanism for chain growth/stoppage for this molecule. One candidate is hindrance; it has been estimated that the portion of a glycogen molecule where hindrance is significant contains a substantial proportion of the total mass of the molecule.[5] Another possibility is that either of the two enzymes have more than one complexation state (as observed for isoamylase[19]), which would have different kinetics.

The first component of the CLD resembles that of starch. The position of the maximum is of interest, this being shown from modeling[15] to lie between X_{min} and $X_0 + X_{min}$ (see SI). This maximum is at DP 3 for mice and 7 for humans; the latter is consistent with literature data[20] for the minimal DP in human glycogen.

The shapes of the second component, arising from the second chain-stopping mechanism, are qualitatively similar in both mice and humans. This suggests the processes in both are the same but with somewhat different rates. One hypothesis is that this mechanism is crowding. The different rates of chain growth and stoppage inferred from the data fitting for the first component would give similar but not identical chain structures as the chains become denser, and thus[5] a similar but not identical rate of stoppage by crowding. Another possibility is that this second component might instead arise from two sets of one or both of the biosynthesis enzymes (see above).

The latter possibility was tested by least-squares fitting the data to a model[15,21] for this biosynthetic process for starch. The model fitting gives, for each postulated enzyme set, X_0, X_{min}, and the ratios of the rates of chain stoppage to that of chain growth, and makes allowance for the presence of more than one set of enzymes. Details of the application of this model to glycogen, and the fitting results are given in the SI. It is found that the model cannot provide a good fit the data for the different features in the observed glycogen CLD, although the starch version of the same model provides a good fit to the features in the entire CLD range for starch, with its multiple enzyme sets, for all the of many species and varieties examined (e.g., refs 21 and 22). This suggests the inapplicability of the hypothesis that there is more than one enzyme set for glycogen.

The only remaining hypothesis consistent with the data is that the second component arises from hindrance. The glycogen system has such simple kinetics that there does not appear to be any realistic alternative. As sketched in Figure 2, crowding dominates chain stoppage in the outer region of the molecule (which has high molecular density), and BE dominates in the low-density innermost region.

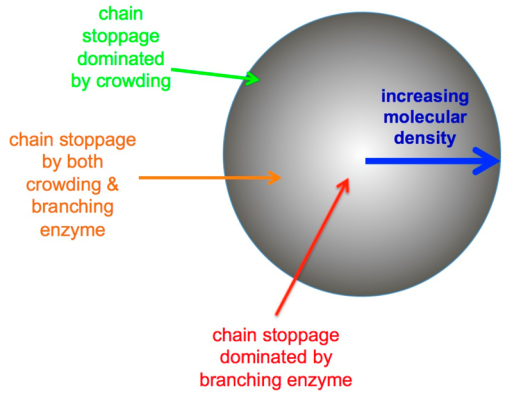

Figure 2. A sketch of the two chain-stopping mechanisms in glycogen.

Note that although it is reasonable to suppose that stoppage by hindrance would give a linear log $N_{de}(X)$, this is not a sufficient test for the applicability of this mechanism, as modeling shows that higher DP regions of the $N_{de}(X)$ calculated with the biosynthesis model with stoppage by BE are very close to linear as well.

The present paper does not consider biological implications of the data, which will be discussed within a much broader study in a later publication.

CONCLUSIONS

The CLDs of liver glycogen of mice and humans, two very different species, indicate two mechanisms for stopping chain growth: branching enzyme and crowding (hindrance). The CLD for hindrance follows simple first-order chain-stopping kinetics. Hindrance not only stops the growth of individual chains but probably of the whole molecule. The inferences obtained here are made possible because of certain unique features in glycogen synthesis and characterization. The slope of the linear region of a plot of log $N_{de}(X)$ for the second component yields the ratio of the rates of chain stoppage to growth.[23] An area for future work is to formulate a quantitative description of the CLD and of the whole-molecule weight and size distributions resulting from this kinetically simple mechanism.

ASSOCIATED CONTENT

Supporting Information

Details of mice and human glycogen sources, experimental details and description and details of the mathematical model can be found in the Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biomac.5b00459.

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Notes

The authors declare no competing financial interest.

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