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Towards a molecular-level theory of carbohydrate processivity in glycoside hydrolases

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Polysaccharide depolymerization in nature is primarily accomplished by processive glycoside hydrolases (GHs), which abstract single carbohydrate chains from polymer crystals and cleave glycosidic linkages without dissociating after each catalytic event. Understanding the molecular-level features and structural aspects of processivity is of importance due to the prevalence of processive GHs in biomass-degrading enzyme cocktails. Here, we describe recent advances towards the development of a molecular-level theory of processivity for cellulolytic and chitinolytic enzymes, including the development of novel methods for measuring rates of key steps in processive action and insights gained from structural and computational studies. Overall, we present a framework for developing structure–function relationships in processive GHs and outline additional progress towards developing a fundamental understanding of these industrially important enzymes.

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Introduction

Structural polysaccharides, such as cellulose and chitin, typically arrange in insoluble, polymeric crystals that form significant components of plant, fungal, and algal cell walls. Microorganisms have evolved suites of enzymatic machinery to degrade these polysaccharides to soluble units for food and energy. These enzyme cocktails are primarily composed of various glycoside hydrolases (GHs) with synergistic functions to efficiently cleave the glycosidic linkages [1]. More recently, additional enzymatic functions beyond the canonical GH enzyme battery have been discovered including oxidative enzymes that selectively cleave glycosidic bonds [2–6]. GH cocktails contain enzymes typically delineated into two broadly defined classes: cellobiohydrolases (CBHs) and endoglucanases (EGs) for cellulose depolymerization, and chitobiohydrolases and endochitinases for chitin depolymerization. EGs are thought to randomly hydrolyze glycosidic linkages primarily in amorphous regions of polymer fibers. Alternatively, CBHs are able to attach to carbohydrate chains and processively hydrolyze disaccharide units from the end of a chain without dissociation after each catalytic event. Processivity is traditionally thought to be a means of conserving energy during enzymatic function, and is a general strategy used in the synthesis, modification, and depolymerization of many natural biopolymers [7]. It is this ability to act processively that imparts significant hydrolytic potential to CBHs from various GH families such as GH Family 6, 7, 18, and 48 and typically makes them the most abundant enzymes in natural secretomes of many biomass-degrading microorganisms. Thus, GHs are the focus of intense protein engineering efforts for the biofuels industry [8•,9].

Here, we aim to briefly summarize developments in understanding GH processivity from the last several years via biophysical, structural, and modeling approaches for several illustrative GH families. In particular, we focus on developments in GH families for which substantial work has been conducted including GH Family 7 and 6, both of which are common fungal cellulolytic enzymes that depolymerize cellulose from the reducing and non-reducing ends, respectively. These systems, along with GH Family 18 chitinases [10], serve as well-characterized models from which a concise theory of carbohydrate processivity that accounts for thermodynamics and kinetics can be developed. This, in turn, will enable the development of more comprehensive structure–function relationships in important biomass-degrading enzymes.

Definitions of GH processivity

Many methods used to determine the degree of processivity, the quantitative approximation of processive ability, describe 'apparent processivity'. The formal mathematical definition of apparent processivity is the number of catalytic events an enzyme performs divided by the number of times the enzyme initiates a processive run, that is, acquires a chain end [11••]. Though a seemingly simple definition, apparent processivity can be difficult to accurately measure, particularly in systems that exhibit biphasic kinetics in their substrate degradation. Additionally, apparent processivity is highly dependent upon the substrate [12••,13••]; thus in practice, apparent processivity can be thought of as the actual processive ability of an enzyme acting on a particular substrate at a given set of conditions. This definition of processivity has utility in comparing degree of processivity across experiments conducted under the same or nearly similar conditions. However, given the variety of methods developed for measuring this quantity and the numerous possible variations in conditions and substrates, comparison of apparent processivity across studies is often not straightforward.

An alternative definition of degree of processivity has emerged describing the theoretical potential for processive ability of GHs, or 'intrinsic processivity' [11••,13••]. Intrinsic processivity is primarily formulated in probabilistic terms and was first developed to describe the processive mechanism of nucleic acid polymerases [14]. McClure and Chow defined steady-state polymerase processivity as a distribution of probabilities defining the likelihood that the polymerase, upon catalysis, will translocate forward rather than dissociate from the newly formed strand. Later, Lucius *et al.* extended this probability-based definition to a kinetic description of helicase action [15]. Kurašin and Väljamäe further extended applicability of this definition to processive GHs, approximating intrinsic processivity as the catalytic rate coefficient, k_{cat}, divided by dissociation rate coefficient, k_{off} [13••], which assumes that for processive enzymes, the probability of dissociation from the substrate is exceedingly low. Using a mathematical formalism, one can consider intrinsic processivity as the limit of apparent processivity as the polymeric substrate approaches ideality. This definition of processivity is potentially advantageous in the development of structure–function relationships, given its direct correlation to measurable kinetic variables and connection to structural features of GH enzymes and substrates.

Methods to examine GH processivity

As GHs are the primary components of cellulolytic and chitinolytic enzyme cocktails, myriad research approaches have been undertaken to understand how their enzymatic cycles occur at the molecular level, including biophysical measurements, structural biology efforts, and various types of modeling. Currently, several standard approaches for measuring apparent processivity have been described, most of which capitalize on the consistent nature of a processive GH product profile. During a processive cycle, GHs primarily produce disaccharides of cellulose or chitin (cellobiose or chitobiose), with relatively few odd-numbered saccharides [16], and thus, an efficient approach to measure apparent processivity for a given enzyme acting on cellulose or chitin is to measure the ratio of disaccharide units produced to the sum of monosaccharide and trisaccharide units. This measurement technique is readily conducted using standard chromatographic methods [17,18]. However, assumptions regarding the initial binding mode, and thus the initial product profile, as well as the presence of intermediate products longer than dimers, can lead to misinterpretation or overestimation of processivity values [19•,20].

A second method for measuring GH processivity involves simultaneously determining the ratio of soluble to insoluble reducing ends [21–25]. Processive GHs produce significantly higher quantities of soluble reducing ends compared to non-processive GHs because they primarily liberate soluble products. To determine the ratio of soluble to insoluble reducing ends, the supernatant and substrate are separately assayed for reducing ends using relatively standard analytical methods. As with the product ratio method described above, this measurement technique also requires assumptions regarding enzyme mechanisms that may bias interpretation of the results. Exo-glycosidases, and a potential preference of endoglycosidases for more easily accessible chain ends, yield soluble reducing ends without processive action. Furthermore, this method is particularly sensitive to the type of substrate used [26], where an abundance of available free chain ends may result in unusually high values of soluble reducing ends from non-processive enzymes.

Recently, new techniques based on substrate labeling have been developed to overcome the limitations presented by the more traditional approaches to measure processivity. One method, termed the single-hit approach, again makes use of the fact that processive enzymes produce more soluble than insoluble reducing ends. In this method, the insoluble reducing end fraction is more accurately quantified through fluorescence-based labeling of reduced cellulose [13••,27,28]. Released soluble reducing ends represent the number of catalytic events, and when reduced cellulose is used as the substrate, the insoluble reducing ends encompass the number of initiation events. The fluorescent labeling of insoluble reducing groups in reduced cellulose allows for visualization of the aldehydes generated upon cellulolytic cleavage. While this method is significantly more accurate than those previously described, it also is not