Sticking to starch

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Harry Brumer[1,2,3,4,*]

From the [1]*Michael Smith Laboratories,* [2]*Department of Chemistry,* [3]*Department of Biochemistry and Molecular Biology, and* [4]*Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada*

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Not all starches in the human diet are created equal: "resistant starches" are consolidated aggregates of the α-glucan polysaccharides amylose and amylopectin, which escape digestion by salivary and pancreatic amylases. Upon reaching the large intestine, resistant starches become fodder for members of the human gut microbiota, impacting the metabolism of both the symbionts and the host. In a recent study, Koropatkin *et al.* provided new molecular insight into how a keystone bacterium in the human gut microbiota adheres to resistant starches as a prelude to their breakdown and fermentation.

In the years since the initiation of worldwide human microbiome projects (1), the importance of the human gut microbiota (HGM) on human nutrition and health has captured the attention of both researchers and the general public alike. On one hand, the ability of the HGM to break down and ferment "dietary fiber"—the recalcitrant complex carbohydrates that evade our own digestive enzymes—has been appreciated for over half a century (2). On the other hand, the application of modern high-throughput omics technologies has shed new light on the tremendous microbiological and molecular complexity of this ecosystem.

Yet, in many respects, the metabolism of the HGM remains something of a black box: complex carbohydrates in, health-promoting metabolites and effectors out. Commensurate with the rise in available (meta)genomic sequence data, biological chemists and structural biologists have recently been highly active in functionally characterizing bespoke protein systems from the HGM that have evolved to capture and saccharify individual components of dietary fiber (3, 4). Through this work, the HGM has been identified as a rich source of new protein and enzyme families, as well as corresponding distinct three-dimensional structures (5).

The recent work of Koropatkin *et al.* on the starch-binding proteins of the anaerobic bacterium *Ruminococcus bromii* comprises an exciting new facet of the structure–function analysis of complex carbohydrate utilization systems from the HGM (6). *R. bromii* is an important "keystone" member of the HGM that can crossfeed other species through the primary degradation of resistant starches (7, 8), that is, the α-glucan polysaccharides that escape hydrolysis by human amylases (9). Notably, different taxa in the HGM organize their carbohydrate-degrading machinery in distinct configurations at the cell envelope, depending in part upon cell architecture (3, 10). *R. bromii* is a monodermic (Gram-positive) member of the phylum Firmicutes that presents its starch-degrading machinery a remarkable and multiprotein complex dubbed the *amylosome* (11). The hallmark of the amylosome is one or more outer membrane–anchored *scaffoldin* proteins comprised of multiple *cohesin* domains, sometimes also in tandem with specific carbohydrate-binding domains. These cohesin domains serve as receptors for diverse glycoside hydrolases and starch-binding proteins that bear complementary *dockerin* domains. The calcium-mediated cohesin–dockerin interaction therefore comprises a dynamic "plug-and-play" system for the assembly of a multipronged starch-degrading apparatus (Fig. 1).

Cerqueira *et al.* (6) noted that the *R. bromii* genome encodes five scaffoldins and 27 dockerin-containing proteins, five of which can be confidently predicted as amylases (starch glycosidases) based on membership in Glycoside Hydrolase Family 13. The remaining 22 comprise a group of proteins of unknown function, 00whose role in the amylosome is obscured by poor sequence similarity. Quantitative proteomics of the amylosome subsequently underscored the importance of one of these, Sas20, as the seventh most abundant protein, preceded only by five predicted multidomain amylases (Glycoside Hydrolase Family 13 members) and one predicted starch-binding protein (comprised of tandem Carbohydrate-Binding Module Family 26, *i.e.*, CBM26, members). In fact,

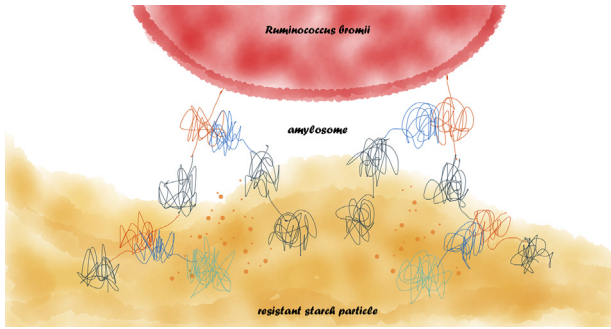

Figure 1. The human gut bacterium *Ruminococcus bromii* adheres to and hydrolyzes the surface of a resistant starch particle *via* the membrane-anchored amylosome. Protein domains of the amylosome are colored as follows: *orange*, cohesins; *blue*, dockerins; *dark gray*, starch binding; and *cyan*, amylases. Malto-oligosaccharides released by amylase activity are depicted as *burnt-orange circles*.

* For correspondence: Harry Brumer, brumer@msl.ubc.ca.

Sas20 consists of two domains of unknown function, Sas20d1 and Sas20d2, separated by a threonine/proline-rich linker and in train with a C-terminal dockerin domain. In addition, Sas20d2 exhibited significant sequence similarity to two tandem domains of unknown function in the scaffoldin, Sca5 (Sca5X25-1 and Sca5X25-2).

Through a detailed modular dissection and biophysical analysis of Sas20 and Sca5, including polysaccharide affinity gel electrophoresis and isothermal titration calorimetry, Cerqueira *et al.* (6) conclusively demonstrated that Sas20d1, Sas20d2, and Sca5X25-2 are indeed novel starch-binding domains. Furthermore, protein crystallography of Sas20d1 and Sca5X25-2 revealed distinct protein folds. The experimental structure of Sas20d1 in complex with maltotriose revealed commonality of the beta-sandwich domain comprising the substrate-binding cleft with starch-binding CBM26 members. Notably, Sas20d1 also contained a distinguishing mobile α-helical domain, which is not observed among CBM26 members and may further stabilize protein–substrate interactions. In contrast, the structure of Sca5X25-2, also in complex with maltotriose, comprised two tandem domains with high structural similarity to domains of SusE and SusF of the *Bacteroides* spp. Starch Utilization System (10). Whereas SusE and SusF bind amylose on the open faces of their respective clefts, dissection of Sca5X25-2 revealed that both homologous domains are required for binding, thus implying a substrate clamping mechanism. A structural homology model of Sas20d2, based on the experimental Sca5X25-2 structure, significantly enabled site-directed mutagenesis, which underscored the essential contributions of individual aromatic side chains in substrate binding by Sas20d1, Sas20d2, and Sca5X25-2.

Finally, small-angle X-ray scattering analyses revealed tremendous intradomain and interdomain flexibility between the β-sheet and α-helical domains in Sas20d1, both beta-domains in Sas20d2, and between Sas20d1 and Sas20d2 themselves. Children and aficionados of the 1980s may appreciate the analogy with the Wacky WallWalker (https://en.wikipedia.org/w/index.php?title=Wacky_WallWalker&oldid=994717139): attachment of *R. bromii* to the much larger particle of resistant starch is mediated by numerous individual and comparatively low-affinity interactions presented on highly flexible structures extending from the cell membrane. The transient nature of these interactions likely enables movement of the associated amylases on the starch surface, while collectively effecting an overall long-lived attachment of the cell to the substrate (Fig. 1).

In the broader context of HGM metabolism, the recent work of Cerqueira *et al.* (6) is particularly revelatory in highlighting the tertiary structural commonality of the starch capture systems across taxa in the absence of strong primary structure similarity. *Viz.*, *R. bromii* has adopted and adapted individual domains with similar architectures to the starch-binding proteins of both *Eubacterium rectale* (phylum Firmicutes; multimodular, CBM-containing amylases) and *Bacteroides thetaiotaomicron* (phylum Bacteroidetes; independent starch-binding proteins SusE and SusF) (10). This author looks forward to further integrative structure–function studies en route to a full molecular understanding of amylosome function.

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Abbreviations—The abbreviations used are: CBM26, Carbohydrate-Binding Module 26; HGM, human gut microbiota.

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