Structure of bifidobacterial sulfoglycosidase revealed the architecture for specific sugar recognition and breakdown of intestinal mucin glycan

Gut microbes have received increasing attention in recent years due to their significant impact on human health. Host animals absorb most of the nutrients in their food through the small intestine. Thus, the carbon sources for bacteria in the colon are poor. Therefore, gut bacteria compete for survival by equipping themselves with various enzymes that degrade food-derived non-digestible carbohydrates that are not absorbed by the host. In contrast, host animals provide nutritional sources for symbiotic microorganisms in another way. The mucus layer of the intestinal epithelium functions as a barrier against pathogens, and its main component, the glycoprotein mucin, serves as an energy source for symbiotic bacteria, helping to prevent the invasion of other microbes. The mucin glycans in the colon are frequently sulfated, and it has been shown that bacterial sulfatase, which hydrolyzes and detaches a sulfate from sugars, is involved in their degradation [1]. We discovered a sulfatase-independent degradation pathway for mucin glycans in *Bifidobacterium bifidum* [2]. Among the bifidobacteria known to elicit various beneficial effects in humans, *B. bifidum* is a prominent species because it contains a repertoire of extracellular glycoside hydrolases (GHs) that can cross-feed liberated sugars and increase the total abundance of *Bifidobacterium* [3]. The key enzyme in the sulfatase-independent degradation pathway is sulfoglycosidase (BbhII), which releases *N*-acetylglucosamine (GlcNAc)-6-sulfate (GlcNAc-6S) from mucin glycans (Fig. 1) [4]. The *in vivo* functions of BbhII in *B. bifidum*-administrated mice as well as in the feces of human infants were also demonstrated [2].

BbhII is a cell wall-anchored extracellular enzyme with a transmembrane region at the C-terminus, consisting of an N-terminal carbohydrate-binding module family 32 (CBM32) domain and a catalytic domain classified as GH family 20 (GH20) (Fig. 2). We solved the crystal structure of BbhII in complex with GlcNAc-6S at 1.65 Å resolution [2]. Diffraction data were collected at the structural biology beamline SPring-8 **BL26B2**, and a dataset from a selenomethionine-substituted crystal was used for phase determination.

The catalytic domain consists of a $(\beta/\alpha)_8$ barrel fold with GlcNAc-6S located at its center. GH20 enzymes use a substrate-assisted mechanism in which the *N*-acetyl group of the substrate acts as a nucleophile in the hydrolysis reaction. The *N*-acetyl moiety of GlcNAc-6S bound to the catalytic site is distorted, making its carbonyl group appropriate for glycosidic bond cleavage (Fig. 3(a)). The distorted *N*-acetyl moiety is supported by the side chains of Y637, D552, and three aromatic residues (W588, W607, and W685). As an acid/base catalyst, E553 forms a hydrogen bond with the O1 hydroxyl group of GlcNAc-6S to facilitate glycosidic bond cleavage. Unexpectedly, no basic residues (e.g., arginine or

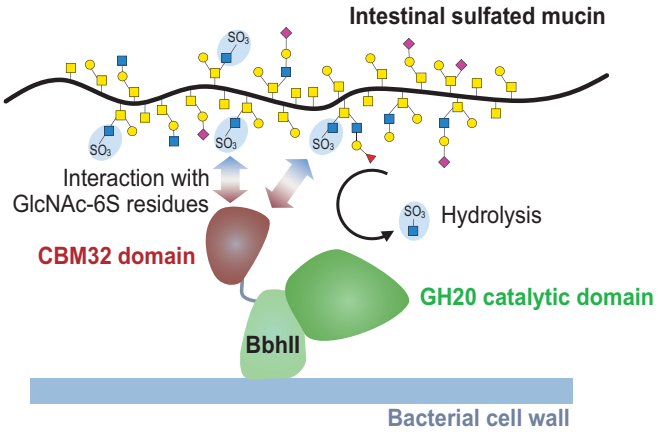

Fig.1. Schematic representation of the CBM-dependent breakdown strategy of mucin glycan by *B. bifidum*. The bacterium interacts with sulfated mucin glycans via the cell-surface-located carbohydrate-binding module family 32 (CBM32) domain of BbhII to initiate glycan degradation using a series of glycoside hydrolases.

lysine) are involved in the recognition of the 6-sulfate group of GlcNAc-6S. Q640, W651, and several water molecules formed hydrogen bonds with sulfate in the active site.

The CBM32 domain consists of a β-sandwich fold containing a Ca^{2+} ion, but the metal was not involved in the glycan binding (Fig. 2). GlcNAc-6S bound through a stacking interaction with the aromatic side chain of W183 and many hydrogen bonds with E62, N89, R95, S97, and N126 (Fig. 3(b)). Again, no basic residues were directly involved in sulfate group recognition. The K_d value toward GlcNAc-6S was 25 μM, indicating a stronger affinity than other CBM32 domains. Although binding to chitosan and β-galactoside has been reported for CBM32s, CBM32 in BbhII was the first to be shown to bind to sulfated glycans.

B. bifidum also contains many other glycosidases on its surface. These extracellular enzymes are expected to capture mucin glycans by CBMs present in the same polypeptide and efficiently release mono- and disaccharides from the glycans, as in the case of BbhII (Fig. 1). Our results provide molecular insights into how gut microbiota utilize carbohydrate-active enzymes to persist in the gut ecosystem, and how these pathways influence microbiome formation in healthy humans.

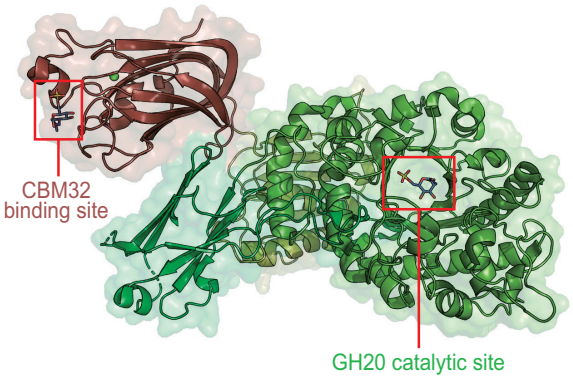

Fig. 2. Overall structure of BbhII. Two GlcNAc-6S-binding sites are indicated. Ca^{2+} bound to CBM32 is shown as a green sphere.

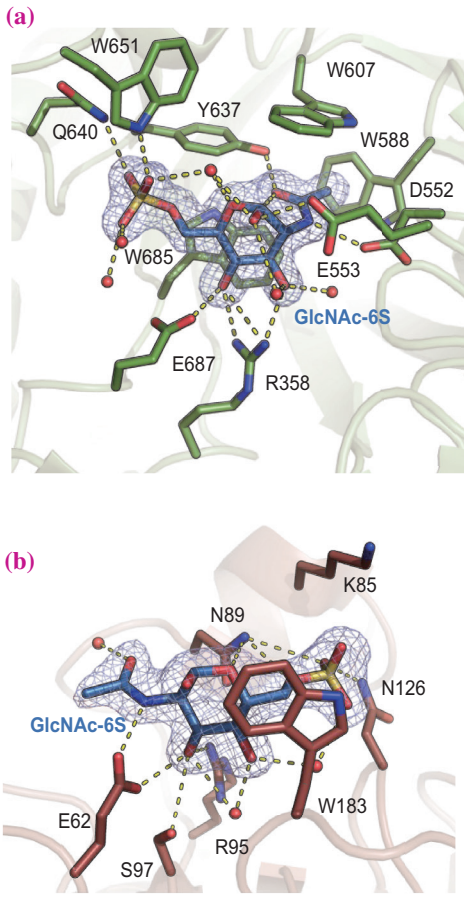

Fig. 3. GlcNAc-6S-binding sites of BbhII. **(a)** The catalytic site. **(b)** The binding site in CBM32.

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