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Article

A Bacterial Homolog of a Eukaryotic Inositol Phosphate Signaling Enzyme Mediates Cross-kingdom Dialog in the Mammalian Gut

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SUMMARY

Dietary $InsP_6$ can modulate eukaryotic cell proliferation and has complex nutritive consequences, but its metabolism in the mammalian gastrointestinal tract is poorly understood. Therefore, we performed phylogenetic analyses of the gastrointestinal microbiome in order to search for candidate $InsP_6$ phosphatases. We determined that prominent gut bacteria express homologs of the mammalian $InsP_6$ phosphatase (MINPP) and characterized the enzyme from *Bacteroides thetaiotaomicron* (BtMinpp). We show that BtMinpp has exceptionally high catalytic activity, which we rationalize on the basis of mutagenesis studies and by determining its crystal structure at 1.9 Å resolution. We demonstrate that BtMinpp is packaged inside outer membrane vesicles (OMVs) protecting the enzyme from degradation by gastrointestinal proteases. Moreover, we uncover an example of cross-kingdom cell-to-cell signaling, showing that the BtMinpp-OMVs interact with intestinal epithelial cells to promote intracellular Ca^{2+} signaling. Our characterization of BtMinpp offers several directions for understanding how the microbiome serves human gastrointestinal physiology.

INTRODUCTION

The adult human gastrointestinal (GI) tract accommodates a bacterial community that comprises trillions of cells. This microbiota has many essential roles in human health (Tremaroli and Bäckhed, 2012): it suppresses proliferation of pathogenic microbes and has important nutritional consequences, including vitamin synthesis and fermentation of complex dietary carbohydrates. Microbial metabolites also regulate the signaling activities of the host's intestinal epithelium, which, for example, aids the development and maintenance of local immune responses. Thus, considerable efforts are now being made to determine the precise nature of the dialog between gut bacteria and the human host. Much of the previous work in this area has focused on the roles of diffusible, small-molecule hormones and nutrients. We now describe a vehicle produced by a prevalent gut bacterium, *Bacteroides thetaiotaomicron* (Bt): a vesicle-enclosed homolog of a mammalian cell-signaling $InsP_6$ phosphatase, MINPP.

Enzymatic homeostasis of $InsP_6$ levels in the gut can have far-reaching consequences for human health. Considerable quantities of $InsP_6$ are ingested daily as it is the primary storage form of phosphorus in cereals and legumes (Kumar et al., 2010). $InsP_6$ is therefore a source of inositol and phosphate, two vital nutrients. However, $InsP_6$ is also considered to have antinutritive properties, given that it inhibits polysaccharide digestibility and chelates divalent cations, thereby limiting their bioavailability in the GI tract (Kumar et al., 2010). More recently, with the emergence of cell-signaling activities for $InsP_6$ and other members of the inositol phosphate family, dietary $InsP_6$ has received separate attention for its anticarcinogenic properties (Fox and Eberl, 2002; Vucenik and Shamsuddin, 2003), at least when present at high concentrations. Conversely, lower concentrations of $InsP_6$ may stimulate tumor cell proliferation (Windhorst et al., 2013). In any case, exogenous $InsP_6$ can enter cells and be metabolized (Windhorst et al., 2013), thereby potentially contributing to intracellular signaling processes in intestinal epithelial and immune cells. For example, $InsP_6$ is the precursor for inositol pyrophosphates, which have pleiotropic functions including the regulation of energy metabolism (Szijgyarto et al., 2011), insulin sensitivity (Chakraborty et al., 2010), and bactericidal activities of immune cells (Prasad et al., 2011).

Humans and other monogastric animals lack enzymes capable of participating in dietary $InsP_6$ homeostasis, relying instead on exogenous phosphatases provided by resident microbes (Haros et al., 2007; Steer et al., 2004). Surprisingly, the nature of the genes that encode these phosphatases

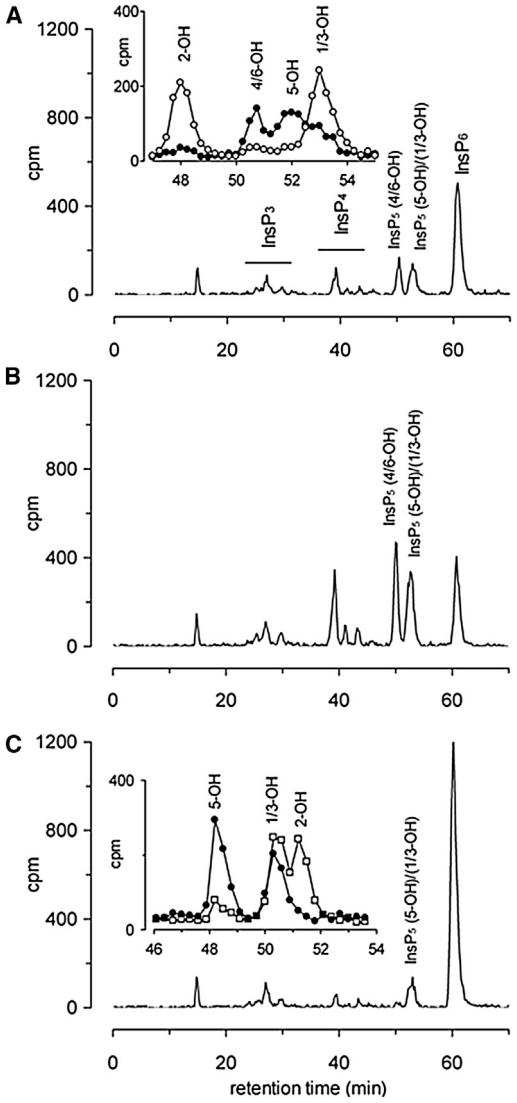

Figure 1. Mutation of Active Site Residues Alters the Specificity of Initial Attack on $InsP_6$ Substrate

(A–C) Products of reaction of native BtMinpp- (A), A31Y- (B), and R183D- (C) substituted enzyme with *myo*-inositol(1,[^{32}P]2,3,4,5,6)P_6 were resolved by Partisphere SAX HPLC. Mutated proteins were incubated at a concentration of 2.5 μg/ml and native protein at 0.25 μg/ml, with 1 mM $InsP_6$. The regions of the chromatogram in which $InsP_3$, $InsP_4$, and specific $InsP_5$ isomers elute are indicated in (A). For native enzyme (inset in A), reaction products were mixed with standards of *myo*-[2-^{3}H]inositol (1,3,4,5,6)P_5 ($InsP_5$ [2-OH]), D/L- *myo*-[2-^{3}H]inositol (1,2,4,5,6)P_5 ($InsP_5$ [1/3-OH]), and D/L- *myo*-[2-^{3}H]inositol (1,2,3,4,5)P_5 ($InsP_5$ [4/6-OH]). Fractions, 0.25 min, were collected and radioactivity was estimated by scintillation counting; ^{3}H, open circles; ^{32}P, filled circles. For R183D-substituted enzyme (inset in C), the reaction products were additionally mixed with standards of *myo*-[^{14}C]$InsP_5$ [2-OH] and D/L- *myo*-[^{14}C]$InsP_5$ [1/3-OH] and resolved on a Adsorbosphere SAX HPLC column. This column separates $InsP_5$ [2-OH] from $InsP_5$ [1/3-OH]. Fractions, 0.25 min, were collected and radioactivity estimated by scintillation counting: ^{14}C, open squares; ^{32}P, filled circles.

is unknown for virtually the entire microbiome of the human GI tract, although they are generally assumed to be "phytases" (Haefner et al., 2005; Steer et al., 2004; Tamayo-Ramos et al., 2012). Such enzymes occur in a number of environmental niches in which they scavenge inorganic phosphate from $InsP_6$, but they have no signaling function. Moreover, if phytases were to be secreted into the GI tract, it is unclear how they might access dietary $InsP_6$ while avoiding digestive proteases. In the current study, our phylogenetic, biochemical, and structural characterization of BtMinpp characterizes an unexpected repertoire of widely distributed and highly active class of $InsP_6$ phosphatases in the GI tract.

RESULTS

B. thetaiotaomicron Express a Homolog of a Mammalian Inositol Phosphate Signaling Phosphatase

The Bacteroidetes are a dominant constituent of the mammalian GI tract microbiota. The genome of *B. thetaiotaomicron* (Xu et al., 2003) contains a gene, *BT_4744*, which has a primary annotation (Hidden Markov Models-based annotation) as encoding a putative multiple inositol-polyphosphate phosphatase (MINPP, EC 3.1.3.62). This is a notable observation because bacteria have not previously been suggested to utilize the inositol phosphate signaling cascade (Michell, 2008). Indeed, MINPPs have only previously been considered to function inside animal cells, mainly by regulating levels of $InsP_5$ and $InsP_6$ (Chi et al., 2000; Romano et al., 1998). Strikingly, BT_4744 shares no significant sequence similarity with the bacterial or fungal phytases described in the literature (see Table S1). We shall refer to the *BT_4744*-encoded enzyme as BtMinpp, and below we further justify this characterization.

High-performance liquid chromatography (HPLC) analysis of the catalytic activity of a His-tagged recombinant form of BtMinpp confirmed that this protein has $InsP_6$ phosphatase activity (Figure 1A). The V_{max} value for $InsP_6$ (178 μmol/mg/min; Table S2) is the highest yet reported for any member of the MINPP family, being several orders of magnitude greater than that for the human (0.006 μmol/mg/min) and avian (0.7 μmol/mg/min) representatives (Ali et al., 1995; Cho et al., 2006). We incubated BtMinpp with Ins(1,[^{32}P]2,3,4,5,6)P_6 substrate and identified the $InsP_5$ products from their HPLC elution positions relative to *myo*-[2-^{3}H]$InsP_5$ standards (Figure 1A, inset). Although Ins(1,3,4,5,6)P_5 ($InsP_5$ [2-OH]) cannot be detected using Ins(1,[^{32}P]2,3,4,5,6)P_6 as substrate, we can be confident that $InsP_5$ [2-OH] is not a major product because of the retention of radiolabel in progressively less-phosphorylated products, $InsP_4$, $InsP_3$, and $InsP_2$ (Figure S1C). In any case, the use of Ins(1,[^{32}P]2,3,4,5,6)P_6 as substrate revealed that multiple $InsP_5$ products were formed by BtMinpp, including D-and/or L-Ins(1,2,3,4,5)P_5 ($InsP_5$ [4/6-OH]), Ins(1,2,3,4,6)P_5 ($InsP_5$ [5-OH]), and D-and/or L-Ins(1,2,4,5,6)P_5 ($InsP_5$ [1/3-OH]) (Figure S2). Such a lack of specificity toward the site of initial attack on $InsP_6$ is a defining characteristic of MINPPs of both plants (Dionisio et al., 2007) and animals (Figure S1A; Ali et al., 1995; Craxton et al., 1997). This catalytic flexibility contrasts with the more precise positional specificity of phytases. In fact, the distinct specificities of different types of phytases are sufficient