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Crystal Structures and Mutagenesis of Sucrose Hydrolase from *Xanthomonas axonopodis* pv. *glycines*: Insight into the Exclusively Hydrolytic Amylosucrase Fold

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Neisseria polysaccharea amylosucrase (NpAS), a transglucosidase of glycoside hydrolase family 13, is a hydrolase and glucosyltransferase that catalyzes the synthesis of amylose-like polymer from a sucrose substrate. Recently, an NpAS homolog from *Xanthomonas axonopodis* pv. *glycines* was identified as a member of the newly defined carbohydrate utilization locus that regulates the utilization of plant sucrose in phytopathogenic bacteria. Interestingly, this enzyme is exclusively a hydrolase and not a glucosyltransferase; it is thus known as sucrose hydrolase (SUH). Here, we elucidated the novel functional features of SUH using X-ray crystallography and site-directed mutagenesis. Four different crystal structures of SUH, including the SUH–Tris and the SUH–sucrose and SUH–glucose complexes, represent structural snapshots along the catalytic reaction coordinate. These structures show that SUH is distinctly different from NpAS in that ligand-induced conformational changes in SUH cause the formation of a pocket-shaped active site and in that SUH lacks the three arginine residues found in the NpAS active site that appear to be crucial for NpAS glucosyltransferase activity. Mutation of SUH to insert these arginines failed to confer glucosyltransferase activity, providing evidence that its enzymatic activity is limited to sucrose hydrolysis by its pocket-shaped active site and the identity of residues in the vicinity of the active site.

Keywords: *Xanthomonas*; carbohydrate utilization locus; sucrose hydrolase; amylosucrase

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Introduction

Plant-associated phytopathogenic bacteria depend on nutrients present in their host plants for survival and growth. Although carbohydrates are the most ubiquitous and abundant carbon sources in plants, little is known about the details of the mechanisms by which phytopathogenic bacteria utilize these carbohydrates. Many bacteria are known to possess a phospho*enol*pyruvate-dependent phosphotransferase system (PTS) for the uptake and concomitant phosphorylation of various sugars.[1,2] In this system, carbohydrate-specific PTS enzymes phosphorylate carbohydrates while they are being translocated into the cytoplasm across the bacterial membrane and, subsequently, the carbohydrates are linked to glycolysis for energy production. PTS-dependent transport and metabolism also occur in *Xanthomonas campestris* pv. *campestris*, a plant pathogen; in this bacterium, fructose is translocated and phosphorylated into fructose-1-phosphate, primarily through the PTS, although a minor and as-yet-identified transport pathway for fructose also operates independently of the PTS.[3,4] Therefore, PTS has served as a model paradigm for carbohydrate transport and metabolism, even in phytopathogenic bacteria.

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Abbreviations used: NpAS, *Neisserea polysaccharea* amylosucrase; SUH, sucrose hydrolase; PTS, phospho*enol*pyruvate-dependent phosphotransferase system; CUT, carbohydrate utilization; Xag, *Xanthomonas axonopodis* pv. *glycines*; SeMet, selenomethionine; PDB, Protein Data Bank.

Recently, a PTS-independent carbohydrate utilization (CUT) system was proposed to exist in *Xanthomonas*.[5] Analysis of the genomic sequences of *Xanthomonas* strains led to identification of a novel type of CUT locus; this locus consists of genes encoding a sucrose-specific transporter at an outer membrane and an amylosucrase-like protein, as well as an inner membrane transporter and a regulator.[5] The presence of a sucrose transporter in *Xanthomonas* implies that *Xanthomonas* also has an enzyme that metabolizes translocated sucrose. Consistent with this metabolic prerequisite, functional analysis has demonstrated that the CUT locus from *Xanthomonas axonopodis* pv. *glycines* (Xag) contains a gene encoding an amylosucrase-like hydrolase that is specific for a sucrose substrate and does not hydrolyze other carbohydrates or their phosphorylated forms.[6] Since the CUT locus encodes a sucrose transporter at the outer membrane, an inner membrane transporter, a sucrose hydrolase (SUH), and a regulator protein, it clearly allows for carbohydrate transport, metabolism, and regulation in pathogenic bacteria, providing a novel type of CUT system that is directly linked to virulence. In addition, a gene cluster utilizing sucrose has also been identified in *Erwinia amylovora*, the causative agent for fire blight of rosaceous plants; this cluster also affects virulence against host plants.[7]

The metabolism of sucrose in CUT-locus-containing *Xanthomonas* begins with the enzymatic action of an amylosucrase-like protein with SUH activity. Typically, amylosucrases belong to glycoside hydrolase family 13 and are transglucosidases (EC 2.4.1.4) containing both hydrolytic and glucosyltransferase activity†.[8,9] These enzymes synthesize an insoluble amylose-like polymer by hydrolyzing sucrose and transferring the product glucosyl units to a growing chain of polymeric glucose.[10–12] However, the amylosucrase-like protein of the CUT locus is unique. Although its sequence is 57% homologous to that of *Neisseria polysaccharea* amylosucrase (NpAS), it hydrolyzes sucrose but has no glucosyltransferase activity and is thus known as SUH (Fig. 1).[6] To date, the other glycoside hydrolase known to hydrolyze sucrose to produce glucose and fructose is an invertase that does not show any sequence homology to SUH and belongs to glycoside hydrolase family 32.[14,15]

In the present study, in order to better understand the exclusive hydrolytic activity of Xag SUH, we determined the crystal structures of SUH and of its substrate and product complexes. Using site-directed mutagenesis and structural comparison with NpAS, we also characterized the structural and functional features of the novel SUH of the CUT locus.

Results and Discussion

Overall structure of SUH

In this study, we determined four different structures of SUH in the absence or presence of substrate or product. These structures represent three discrete conformations that might be observed along the SUH reaction coordinate with a sucrose substrate: native SUH in the presence of a Tris molecule, the E322Q–sucrose complex for the enzyme–substrate Michaelis complex, and the E322Q– and SUH–glucose complexes for the enzyme bound with product. E322Q mutant is a catalytically inactive Xag SUH. Analysis of the highly similar sequences of SUH and other proteins of glycoside hydrolase family 13 suggested that Glu322 is the general acid/base in this hydrolase (Fig. 1).[16] Each structure provides a snapshot of the stereochemical features occurring along the catalytic pathway. Although some local and global differences between the structures were found, as discussed below, the overall topology of SUH in these structures was essentially unchanged.

Figure 2 shows the overall structure of the E322Q–sucrose complex. Its central domain is a $(\beta/\alpha)_8$-barrel, and four additional domains are attached to the barrel fold. The structure is oriented such that the C-terminal ends of the eight parallel β-strands in the barrel are perpendicular to the plane of the figure. Two of the other domains (B and B′; see below for nomenclature) are formed independently by the long extruded loops between the β-strands and α-helices in the barrel and sit on top of the C-terminal ends of the parallel barrel β-strands. The all-helical N-terminal domain and the exclusively β-stranded C-terminal domain are located on the opposite sides of the barrel fold, where they interface with the outer α-helices of the central $(\beta/\alpha)_8$-domain.

Consistent with the high sequence similarity between SUH and NpAS, the observed five-domain structure of SUH is essentially identical with that of NpAS; the 559 equivalent C^{α} atoms of native SUH–Tris complex and NpAS in the absence of a ligand [Protein Data Bank (PDB) ID: 1G5A][17] have a root-mean-square deviation (r.m.s.d.) of 1.78 Å. For these reasons, we adopted the NpAS domain nomenclature for SUH (Fig. 1). Specifically, we designated the central $(\beta/\alpha)_8$-fold as the A-domain, the long extrusion between Aβ3 and Aα3 as the B-domain (residues 180–255), the region between Aβ7 and Aα7 as the B′-domain (residues 394–461), and the N- and C-terminal domains as the N- and C-domains. In particular, domains A, B, and C are common structural elements usually found in glycoside hydrolase family 13, although there are large variations in length, sequence, and even tertiary structure on B-domain.[18]

Active site of SUH

The location of the active site was suggested by the bound Tris molecule, that is, 2-amino-2-hydroxymethyl-1,3-propanediol from the buffer solution, in the native SUH structure. Tris molecule was known to be an inhibitor of α-glucosidase and some of glycoside hydrolase family 13 enzymes.[19,20] The substrate or product binding site was confirmed in the structures complexed with ligand. The active site is located at the region composed of the C-terminal

† www.cazy.org/fam/GH13.html