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Structure and biochemical characterization of bacteriophage phi92 endosialidase

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ABSTRACT

Surface-associated capsular polysaccharides (CPSs) protect bacteria against phage infection and enhance pathogenicity by interfering with the function of the host innate immune system. The CPS of enteropathogenic *Escherichia coli* K92 is a unique sialic acid polymer (polySia) with alternating α2,8- and α2,9-linkages. This CPS can be digested by the gene 143 encoded endosialidase of bacteriophage phi92. Here we report the crystal structure of the phi92 endosialidase in complex with a dimer of α2,9-linked sialic acid and analyze its catalytic functions. Unlike the well characterized and homologous endosialidase of phage K1F, the phi92 endosialidase is a bifunctional enzyme with high activity against α2,8- and low activity against α2,9-linkages in a polySia chain. Moreover, in contrast to the processive K1F endosialidase, the phi92 endosialidase degrades the polymer in a non-processive mode. Beyond describing the first endosialidase with α2,9-specificity, our data introduce a novel platform for studies of endosialidase regioselectivity and for engineering highly active α2,9-specific enzymes.

Introduction

The cellular envelope of Gram-negative bacteria is characterized by K- and O-antigens that are formed by lipid-linked or free surface polysaccharides. In many cases, these molecules create a thick continuous layer that fully covers the cell surface and is called a capsule (Whitfield, 2006). In bacteria causing meningitis, pneumonia, and septicemia, the complexity of K- and O-antigen organization and the thickness of the capsule have been shown to correlate positively with pathogenicity (Cross, 1990; Taylor and Roberts, 2005). Due to their size and charge, capsular polysaccharides (CPSs) shield the bacterium from the immune system. Moreover, CPSs of some bacterial pathogens are identical to saccharides produced by mammalian cells, which confers protection by molecular mimicry (Finne et al., 1983) such as the case for the neuroinvasive bacteria *Neisseria meningitidis* (*Nm*) serogroup B and *Escherichia coli* K1. In both organisms the capsule consists of α2,8-linked polysialic acid (polySia), a structure that is also abundant on the surface of many human cells (α2,8-linked polySia will henceforth be described as K1-polySia) (for review see (Muhlenhoff et al., 2013)).

Besides their protective functions, the CPSs serve as receptors for specialized bacteriophages (Lindberg, 1977; Scholl et al., 2005). To gain access to the membrane and infect an encapsulated bacterium, a phage must degrade the CPS (Sutherland, 1977; Scholl and Merril, 2005). In phages that infect polySia-encapsulated bacteria, the degrading enzymes are endosialidases (or endo-N-acetyl-neuraminidases, endoN) (Gross et al., 1977; Leiman et al., 2007) and are present on the particle in the form of tailspikes or tail fibers (Stirm et al., 1971).

About thirty genomes of *E. coli* K1-specific phages have been characterized so far and all contain a gene that encodes an endoN (Jakobsson et al., 2012). The tailspike of coliphage K1F (endoNF) serves as a paradigm for all these enzymes as its function, folding properties, and crystal structure in the apo- and substrate-bound form have been well characterized (Mühlenhoff et al., 2003; Stummeyer et al., 2005; Schwarzer et al., 2007, 2009; Schulz et al., 2010b, 2010c). The catalytic part of endoNF is a mushroom-shaped homotrimer with each subunit comprising three distinct domains: an N-terminal β-propeller found in all sialidases with an inserted nine stranded β-barrel domain, and a C-terminal β-helix domain unique to phage proteins. The β-propeller and β-barrel domains form the cap of the mushroom and the β-helical domain comprises its stalk. Roughly in the middle, the triple β-helix is interrupted by a small triple β-prism region (Stummeyer et al., 2005). Folding of the trimeric endoNF involves auto-proteolytic release of its C-terminal chaperone domain in a Ser–Lys dyad reaction

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(Mühlenhoff et al., 2003; Schwarzer et al., 2007; Schulz et al., 2010a; Schulz and Ficner, 2011). The release of the chaperone domain increases thermal stability of endoNF (Schwarzer et al., 2007) and unmasks a polySia binding site located in the stalk domain conferring processivity to the enzyme (Schwarzer et al., 2009; Jakobsson et al., 2012).

Sequences of all known endoN proteins are very similar and all the functionally characterized enzymes specifically cleave α2,8-linkages in a sialic acid polymer. However, polySia with other type of linkages exist. Of particular importance is α2,9-linked polySia that forms the capsule of *N. meningitidis* serogroup C (NmC-polySia). Another example is enteropathogenic *E. coli* K92, whose polySia capsule contains alternating α2,8 and α2,9 linkages (K92-polySia) (Furowicz and Ørskov, 1972; Egan et al., 1977; Kwiatkowski et al., 1983). Recently, we have characterized bacteriophage phi92 that infects *E. coli* K92. It contains an endosialidase gene (gene *143*) and carries endoN92 enzymes on the phage particle (Schwarzer et al., 2012). Taking into account that the substrate of endoN92 is different to that of all previously studied endosialidases, we set our goal to characterize this enzyme functionally and structurally.

Here we report the crystal structure of the catalytic fragment of endoN92 (residues 76–756) in complex with its major cleavage product, a dimer of α2,9-linked sialic acid (α2,9-DP2; DP stands for degree of polymerization), demonstrate that endoN92 has a capacity, albeit very low, to cleave α2,9-linkages and present a comparison of biochemical and biophysical properties between endoN92 and endoNF.

Results

Phylogenetic analysis of the endosialidase 92 (endoN92)

All putative endoN sequences available at the GenBank (see alignment in Fig. S1) can be grouped into four separate phylogenetic clades – I through IV, Fig. 1. EndoN92 (a myovirus enzyme) is unique and represents the only member of clade I. Sequences from siphophages 63D, K1G and K1H form clade II and clade III contain endosialidases that are encoded in prophages and genetic islands of *E. coli* K1 strains. This clade splits into two classes – IIIa and IIIb – with in-class sequence similarities close to 100% (Table S1). The well-studied endoNF is in clade IV, which also contains other functionally characterized podophage-derived endosialidases like endoNA (Jakobsson et al., 2007), endoNE (Gerardy-Schahn et al., 1995), and endoNK1-5 (Scholl et al., 2001). The sequence alignment (Fig. S1) together with previous work on endoNF and endoNE (Mühlenhoff et al., 2003; Stummeyer et al., 2005) demonstrated that all family members exhibit a conserved domain organization and consist of an N-terminal particle-binding domain, a large central catalytic part comprising several domains, and a relatively small C-terminal chaperone domain. Because the N-terminal particle-binding domains vary considerably in terms of size and sequence, the phylogenetic analysis was repeated in the absence of these sequences. The resulting tree had shorter branches, but segregation into clades remained identical to Fig. 1 (data not shown).

To further investigate functional and structural motifs, the endoN92 sequence was aligned to the best-characterized representative of each clade (Fig. 2). The six-bladed β-propeller sialidase domain (marked by an open box in Fig. 2) and the β-barrel domain (zigzag box in Fig. 2) of the catalytic part were found to comprise the region of the highest sequence conservation. The β-propeller domain contains the active site of the enzyme and the β-barrel domain displays the polySia binding site b1, which could be essential for directing the polymeric substrate into the active site. In contrast, the second polySia binding site b2 in the β-helical stalk domain of endoNF is not conserved. Because the site b2 determines the processivity of substrate degradation by endoNF (Schwarzer et al., 2009), weaker sequence conservation at this position suggests that processivity could be a specific feature of

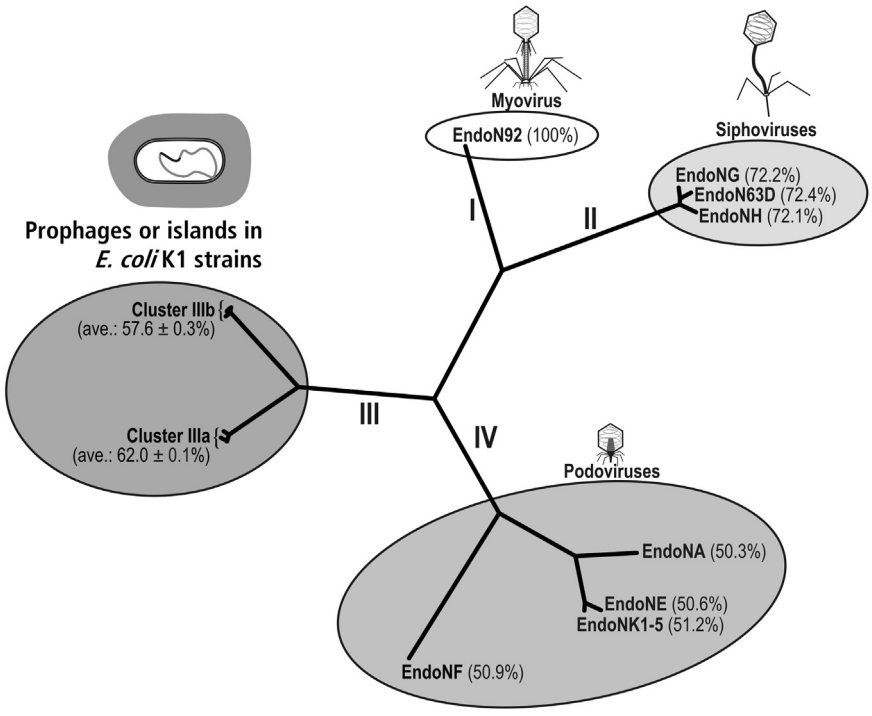

Fig. 1. Phylogenetic tree of endosialidases. The tree was generated based on the alignment shown in Fig. S1. Endosialidases from phages of the same family (schematically shown above) are *shaded*. Sequence similarities to endoN92 are given in *brackets*. The four apparent clades correspond to the endosialidases from myoviruses (I), siphoviruses (II), prophage-related (III), and podoviruses (IV). The prophage clade can be further subdivided into two independent groups (IIIa and IIIb) that each comprise seven closely related endosialidases from different prophages.

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