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X-ray Structure of Catenated Lytic Transglycosylase SltB1

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Abstract

Formation of catenanes by proteins is rare, with few known examples. We report herein the X-ray structure of a catenane dimer of lytic transglycosylase SltB1 of *Pseudomonas aeruginosa*. The enzyme is soluble and exists in the periplasmic space, where it modifies the bacterial cell wall. The catenane dimer exhibits the protein monomers in a noncovalent chain-link arrangement, whereby a stretch of 51 amino acids (to become a loop and three helices) from one monomer threads through the central opening of the structure of the partner monomer. The protein folds after threading in a manner that leaves two helices ($\alpha 1$ and $\alpha 2$) as stoppers to impart stability to the dimer structure. The symmetric embrace by the two SltB1 molecules occludes both active sites entirely, an arrangement that is sustained by six electrostatic interactions between the two monomers. In light of the observation of these structural motifs in all members of Family 3 lytic transglycosylases, catenanes might be present for those enzymes, as well. The dimeric catenane might represent a regulated form of SltB1.

Catenation in proteins is observed only rarely, with a handful of known examples.¹ Most involve covalent intramolecular disulfide bonds that mechanically interlock two protein molecules.^{2,3} The bacteriophage HK97 capsid structure is the classical sole example for a covalent isopeptide bond between two amino acid side chains, maintaining a chainmail arrangement.⁴ A few examples involve noncovalent annular polymeric quaternary structures of proteins that lock into one another in the course of the supramolecular assembly.^{5–8} To the best of our knowledge, an example of two (or more) protein molecules embracing each other by noncovalent interlocking of loops does not exist. We describe herein the chance discovery of one such example.

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Supporting Information

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The authors declare no competing financial interest.

The members of the superfamily of bacterial lytic transglycosylases (LTs) fragment the peptidoglycan, the primary constituent of the cell wall, by a nonhydrolytic reaction.^{9,10} The backbone of the peptidoglycan is comprised of repeats of the disaccharide *N*-acetylglucosamine (NAG)-*N*-acetyl-muramic acid (NAM). A unique pentapeptide is appended to the NAM unit, which is the site of cross-linking with neighboring strands of peptidoglycan. The reactions of the LTs lead to the formation of the 1,6-anhydromuramyl moiety (anhNAM), which is the hallmark of this enzymatic transformation (Figure 1A). In the course of our characterization of the reactions of the 11 known lytic transglycosylases (LTs) of *Pseudomonas aeruginosa*,¹¹ we set up crystallization trials for SltB1. This is a soluble (non-membrane-anchored) LT, which is capable of performing both the endolytic (cleavage in the middle of a strand of peptidoglycan) and exolytic (fragmentation from one terminus) reactions. However, SltB1 is primarily exolytic.¹¹ The polymeric peptidoglycan occupies a minimum of four sugar-binding subsites in the enzyme active site, straddling the seat of reaction (Figure 1A). The enzyme promotes formation of a transient oxocarbenium species, which entraps the C₆ hydroxyl of the NAM unit, en route to the formation of the anhNAM moiety.

A crystal structure for SltB1 was first reported by Nikolaidis et al.¹² (Figure 1B). SltB1 presents three domains: an N-terminal domain (domain 1), a catalytic domain that resembles the fold of goose-type lysozyme, and a C-terminal domain (domain 2), colored yellow, salmon, and magenta, respectively (Figure 1B). Domain 1 is comprised of five helices, the first three (α 1– α 3) from the N-terminal region (residues 40–78) and the remaining two (α 8 and α 9) spanning amino acids 143–188. Domain 2 (residues 242–307) presents both α -helices and a β -sheet. The catalytic domain is sandwiched between domains 1 and 2 and presents an EF-hand-like motif containing a Ca²⁺ ion displaying bipyramidal coordination.¹²

During our efforts to crystallize SltB1, we could not reproduce the crystallization conditions reported previously, to which we will return later.¹² Hence, we broadly screened for a suitable crystallization condition. SltB1 was finally crystallized by the hanging drop method in 1 M sodium citrate and 0.1 M sodium cacodylate (pH 6.5), and the X-ray data were processed at 2.5 Å resolution with excellent statistics (Table 1). Two monomers were found in the asymmetric unit showing the electron density for the complete peptide chains (Figure S1A). Our structure of monomeric SltB1 is essentially identical to the previously reported structure, inclusive of the calcium ion-binding site. Differences with regard to the peptide backbones were a complete definition of all the amino acids of domain 2 (three amino acids were invisible in the previous structure), a displacement (2.4 Å) for domain 2 between the two structures, a slight change in the bridge loop (residues 78–86), and, finally, the fact that three more amino acids were seen in the N-terminal portion of our structure (invisible in the previous one). Notwithstanding the profound similarities of the backbones of the structure of SltB1 by Nikolaidis et al. and that of ours, our structure was that of a homodimer catenane as a point of distinction. This catenane structure does not involve creation of covalent bonds to form a cyclic protein as in the bacteriophage HK97 capsid structure, but the formation of two interlocked dimers resembling the links of a chain. Interestingly, this dimer is not formed by three-dimensional domain swapping¹³ in which proteins exchange their identical domains but the two peptide backbones of the asymmetric unit (monomers A and B) were interlocked with the bridge loop of one monomer ensconced within the active-site groove of

the other (Figure 1D). The main causes of dimerization by three-dimensional domain swapping are a change in pH, a change in temperature, mutation in the protein, the presence of denaturants, and binding of a ligand.¹⁴ None of these apply for the case of SltB1. The electron density map for the entire catenated SltB1 and, in particular, that for the two interlocked bridge loops were of high quality, ruling out any misinterpretation of the density model (Figure S1B). In this symmetric arrangement for the two protein monomers, the bridge loop of each protein entirely blocks the active site of the partner protein. An extensive network of electrostatic interactions between the two protein monomers stabilizes the catenane (Figure 1E). Hydrogen bonds and salt-bridge interactions are established between the amino acids within the bridge loop and $\alpha 3$ and those of the catalytic groove of the partner (Figure 1E). These interactions include a salt bridge between the catalytic Glu135 of one monomer and Arg79 of the partner protein.

The X-ray structures of monomeric SltB1 and its dimeric catenane bring forth the question of how these structures could interchangeably form. The need for conformational flexibility of SltB1 is self-evident in light of the nature of the embrace between the two proteins. Indeed, the catenane cannot form without opening of the bridge loops of both proteins in a dramatic fashion. This would require the first three N-terminal helices ($\alpha 1$ – $\alpha 3$) together with the bridge loop (which links these helices to the catalytic domain) to unfold and to separate from the rest of the protein. Helices $\alpha 1$ – $\alpha 3$ interact with two helices within the catalytic domain essentially by hydrophobic interactions (Figure 2), and only a few polar interactions are observed. Congruent with the required movement of this region in unfolding, the highest *B* factors seen for the SltB1 monomer are concentrated at helices $\alpha 1$ and $\alpha 2$ and the bridge loop (Figure S2). In the catenane structure, helices $\alpha 1$ and $\alpha 2$ serve as “stoppers”, akin to the butterfly back of an earring, which prevent the backbone of SltB1 from disengaging from the embrace seen in the catenane; for the reversal of catenane formation to take place, at a minimum, helices $\alpha 1$ and $\alpha 2$ must unravel in both peptide chains.

It is noteworthy that the members of the Family 3 LTs share a high degree of structural similarity, which make us wonder if the observation of the catenane form for SltB1 might also have implications for the existence of catenane forms for other members. The structures of two of these enzymes, MltB (a soluble truncated version is also known as Slt35) of *Escherichia coli*^{15–18} and SltB3 of *P. aeruginosa*,¹⁹ present shared features with that of SltB1 (Figure S3). They all have (i) similar catalytic domains, sandwiched between domains 1 and 2 (SltB3 possesses an extra peptidoglycan-binding domain, annotated PG_binding_1, Pfam PF01471), (ii) a Ca²⁺-binding loop within the catalytic domain, (iii) an active-site groove positioned adjacent to the bridge loop (in the Slt35 structure, part of the loop was not visible in the electron density map because of its high mobility), and (iv) the same three mobile helices observed at the N-terminal region mainly connected by hydrophobic interactions with domain 1. Analyses of the *B* factors for these structures also revealed that the highest *B* values are concentrated in the same regions seen in the SltB1 structure (data not shown), consistent with their inherent mobility. Thus, all structural features for catenane formation would appear to be shared in known Family 3 LTs.

An important question is whether catenation of SltB1 is a biologically relevant phenomenon, or a mere artifact of our experiments. We admit that a definitive answer is not at hand, but the following observations are worthy of consideration. The conditions used previously by Nikolaidis et al.¹² in crystallizing monomeric SltB1 did not work in our hands, suggesting that catenation had already taken place before the crystallization trial had been set up (perhaps because of temporal differences in the handling of the concentrated protein by the two research groups); in other words, the catenane is likely not an artifact of high concentrations of SltB1 used to grow crystals (especially so, as our crystals were obtained at a protein concentration, 13.7 mg/mL, lower than 20 mg/mL, which was used by Nikolaidis et al.). SltB1 is the most active (considering the total muropeptides released from the sacculus) among pseudomonal LTs¹¹ and, together with the other Family 3 LTs, efficiently turns over cross-linked peptidoglycan chains.¹¹ In this sense, opening of the bridge loop in SltB1 (and other Family 3 members) would unmask the active site, allowing the observed turnover of cross-linked chains. Activities of LTs are regulated. The presence of additional domains of unknown functions in various LTs, which have been presumed to serve roles in the formation of complexes with other proteins, is suggestive of this regulation. For example, SltB1 is known to bind to penicillin-binding protein 2, which is anchored to the inner membrane.²⁰ We note also documentation of allostery in regulation of MltF of *P. aeruginosa* involving a domain that binds to muropeptides, cell wall-derived natural products, which facilitates a large conformational change that takes the active site from an occluded conformation to an open one.²¹ The notion that a highly active soluble LT such as SltB1 would freely diffuse in the periplasm is refuted. Hence, the catenane structure could in principle serve a regulatory function in that the active sites in both catalytic domains are fully occluded and cannot participate in catalysis (an inactive form). The monomeric version would represent active SltB1. What triggers this conversion is not known at present. The trigger could be interactions with partner proteins or with muropeptides, which serve as signaling molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (A) Exolytic reaction of SltB1 with the four subsites marked. (B) Three-dimensional structure of SltB1 of *P. aeruginosa* as reported by Nikolaidis et al.¹² The catalytic domain (salmon ribbon) is flanked by domains 1 and 2 (colored yellow and magenta, respectively). The catalytic residue is labeled with an asterisk and is shown as capped sticks. (C) Superimposition of the structures of SltB1 as reported by Nikolaidis et al. (green) and in this work (salmon). (D) Stereoview of the catenated SltB1. One monomer is represented by its Connolly solvent-accessible molecular surface (salmon) and the other by gray ribbons. (E)

Stereoview showing the interactions stabilizing the catenane. Relevant residues are shown as capped sticks and labeled. The color scheme is the same as that in panel D.

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Figure 2.

(A) Interactions between helices $\alpha1$ and $\alpha2$ and the other two helices of domain 1. The interactions mainly occur via hydrophobic residues, and very few hydrogen bond interactions are present. Residues involved in interactions are represented as capped sticks. The bridge loop and helices $\alpha1$ - $\alpha3$ are colored dark red, and the remaining protein is colored salmon.

Table 1.**Data Collection and Refinement Statistics for SlTB1**

Data Collection ^a	
space group	<i>C</i> 2
cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	115.06, 116.36, 54.87
α , β , γ (deg)	90, 118.41, 90
wavelength	0.9800
resolution (Å)	48.26–2.50 (2.64–2.50)
<i>R</i> _{pim}	0.05 (0.51)
CC _{1/2}	0.99 (0.65)
mean <i>I</i> / σ <i>I</i>	11.4 (1.7)
completeness (%)	99.9 (99.8)
redundancy	6.7 (6.6)
Refinement	
resolution (Å)	48.26–2.50
no. of reflections	21993
<i>R</i> _{work} / <i>R</i> _{free}	0.18/0.23
no. of atoms	
protein	4833
ligands	5
ions	2
water	74
root-mean-square deviation	
bond lengths (Å)	0.003
bond angles (deg)	0.838
Protein Data Bank entry	5O8X

^aValues in parentheses are for the highest-resolution shell. One crystal was used to determine each structure.