Solving a Cell Division Puzzle in Bacteria Antibiotic Discovery Potential

Multiple antibiotic resistant bacteria have been the subject of newspaper articles and government reports recently (1, 2). At NMBU in Ås, Daniel Straume, Gro Anita Stamsås and Leiv Sigve Håvarstein have recently published an article in Nature Communications (3) that suggests a new avenue for antibiotic discovery. To complete the research, the team had to solve puzzling results in earlier reports about cell division in Streptococcus pneumoniae.

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It had been suspected for several years that the PcsB protein in Streptococcus pneumoniae is involved in cell division because its CHAP domain had been shown in other bacteria to be able to cleave peptidoglycan (PG) residues in cross walls holding daughter cells together after cell duplication. In spite of this, repeated attempts to demonstrate PG hydrolase activity in PcsB were unsuccessful. Researchers at NMBU decided to use a structural approach to try to solve this puzzle in collaboration with x-ray crystallography specialists in Madrid, Spain.

Gro Anita Stamsås, Daniel Straume and Leiv Sigve Håvarstein.

The first challenge was to purify enough of the PcsB protein for x-ray crystallography. Using a concept developed earlier involving choline tags (4, 5), Stamsås et al. (6) constructed a fusion gene for PcsB consisting of six histidine residues on the N-terminus followed by a choline-binding domain from the Streptococcus gene cbpD followed by a proteolytic site for TEV protease and the PcsB structural gene. This construct was then expressed in E. coli using the lacT7 promoter and then the PcsB protein was purified by the following steps: 1) the bacterial extract from a 4 hour culture was applied to a DEAE-cellulose column where the choline-binding tag bound to the DEAE component of the column matrix, 2) the column was washed with high salt to elute proteins bound ionically to the DEAE-cellulose, 3) choline-tagged PcsB was then eluted with 0.14 M choline, 4) eluted protein was subjected to further purification using immobilized metal affinity chromatography and, finally, 5) the PcsB protein was cleaved from the choline-binding tag with TEV protease.

The purification protocol yielded more than 5 mg of fusion protein per liter of culture medium with better than 95% purity. A second advantage of the protocol was that the choline-binding tag seemed to improve the solubility of PscB. Other researchers had found earlier that expression of native PcsB in bacteria usually resulted in aggregates that were difficult to solubilize.

Analysis of PcsB crystals in Spain gave uncertain results. As Daniel Straume explains it, 'The best they could tell us in the beginning was that the crystals looked like spaghetti and meat balls with the spaghetti being coiled-coil domains and the meat balls being the CHAP domains. They couldn't put the structure together.'

As a way to solve the phase problem associated with structure determination of native PcsB, the NMBU group re-engineered PcsB by adding two new methionine residues to the sequence, raising the total number of methionines in the protein to four. They then produced a Se-met version of the PscB fusion in E. coli and purified it using their choline-binding tag.

X-ray crystallography (Fig. 1) of Se-met PscB gave clear evidence for a dimeric PscB structure in which V-shaped coiled-coil domains of each monomer act as a pair of tweezers locking the CHAP domains of each partner into inactive configurations. So, the reason that PG hydrolase activity had not been detected with PscB before had to do with the dimer's structure.

Does the CHAP domain of PscB, itself, have inherent PG hydrolase activity? To answer this question, PscB CHAP was produced independent of the rest of the protein and tested for enzyme activity. It was enzymatically active. Next, PscB CHAP was swapped for the CHAP domain of another protein (LytF, reference 7) for which PG hydrolase activity had been demonstrated. As expected, the LytF-PscB CHAP fusion protein had PG hydrolase activity.

Putting together these new findings with information about the complex of proteins

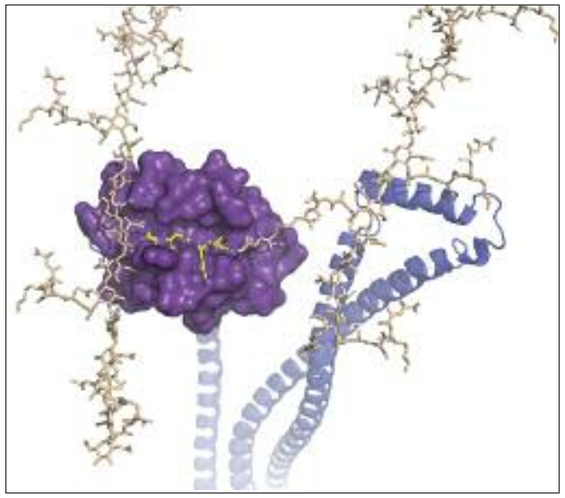

Fig. 1. Crystal structure of the PcsB peptidoglycan (PG) hydrolase in association with its PG substrate

associated with PcsB at the PG septum in dividing Streptococcus pneumoniae cells, the Nature Communications paper presents a model (Fig. 2) with the PcsB from both daughter cells form the inactive dimer in the septum. PcsD is located in the PG matrix, held in place by FtsX which, in turn, is bound to the FtsE ATPase in the cytoplasm. ATP hydrolysis in the cytoplasm by FtsE could shift the orientation of CHAP domains in PcsB, activating their hydrolysis of PG.

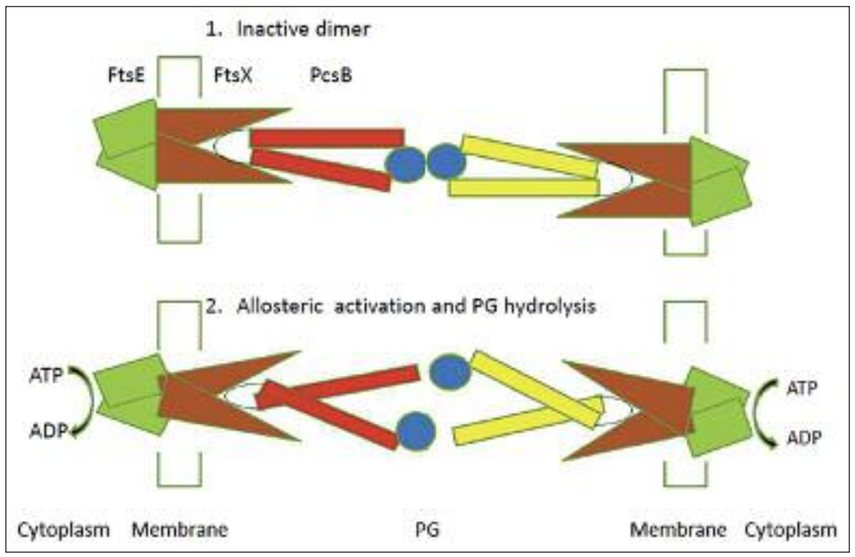

Fig. 2. Model for regulation of PcsB hydrolytic activity in the bacterial cell division septum. The inactive dimer is located at the septum by interaction with FtsX. ATPase activity of FtsE induces an allosteric change in PscB through FtsX which is located in the membrane (box), causing the exposure of the CHAP domains (blue balls) and degradation of PG. This Figure is based on results in Bartual et al. 2014 (ref. 3).

Given the fact that Streptococcus pneumoniae is a serious human pathogen and that these findings characterize a critical enzyme involved in its cell division, it's interesting to speculate that PscB could be the target for discovery of new antibiotics. Use the PG hydrolase assay with CHAP to screen thousands of chemicals on microtiter plates, using a robot to try to find chemicals that block CHAP activity and, therefore, also block growth of Streptococcus pneumoniae. When NBS Nytt asked Leiv Sigve Håvarstein about this possibility, he said, 'It's an interesting idea but it would cost a lot more than we have at this point. The two drawbacks I see with this approach are 1) the assay needs to be made more quantitative and 2) these types of screens are notorious for producing false positives.'

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