

Smart superglue in streptococci? The proof is in the pulling

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Gram-positive bacteria use reactive thioester-containing proteins to form covalent bonds, which may enable strong adhesion to host surfaces, but how these proteins selectively adhere to different surfaces is not clear. The Editors' Pick by Echelman *et al.* applied single-molecule force spectroscopy to show that an adhesin protein can regenerate its thioester in the absence of pulling. This selective interaction would represent a new principle of mechanical proof-reading, whereby only reactions supporting anchorage of the bacterium are maintained.

For bacteria to colonize a host, they usually must establish strong association with host surfaces, with important consequences from antibiotic-resistant biofilms on medical implants (1) to *Yersinia*-flea interactions in bubonic plague (2). Investigation of the molecular basis of such adhesion has revealed new principles of biomolecular function, including regulated amyloid formation, catch bonds (where interaction becomes more stable with mechanical force) (3), and new protein cross-links (4). It has recently been discovered that many Gram-positive bacteria encode proteins containing thioesters (Fig. 1), which may form covalent bonds to target surfaces (5, 6). A new study by Echelman *et al.* (7) sheds important new light on the sophistication of this anchoring.

Streptococcus pyogenes has pili only ~2 nm wide but more than 1 μ m in length, which are important for host interaction (4). The body of the pilus comprises repeated units of the major pilin protein (Spy0128), which is locked together with spontaneous isopeptide bonds (4), making the protein fold highly resistant to stretching (8). Pili are covalently linked to the peptidoglycan cell wall of the bacterium, whereas at the distal end of the pilus is the Spy0125 protein (5). Spy0125 is a thioester-containing protein, and so it has been hypothesized that this thioester could be the "superglue" that enables the pilus to form a covalent bond with the host, to achieve extremely force-resistant anchoring. The best known thioester-containing proteins are in the mammalian complement pathway, where protease cleavage unveils the thioester for promiscuous labeling of nearby surfaces, promoting scrutiny and destruction of pathogens by the immune system (9). Because Spy0125 is not known to be activated by proteolysis, a key question is how is the thioester's reactivity controlled? Is reaction only about proximity, where non-covalent docking positions a nucleophilic amino

acid from a host's receptor adjacent to Spy0125's thioester, or is there another mechanism to ensure selectivity?

Echelman *et al.* (7) investigated these questions using single-molecule force spectroscopy to pull on domains of Spy0125. Protein domains can be stretched in a defined direction through anchoring via cysteine residues to the atomic force microscope tip. Domains are seen to unfold one-by-one, giving information on the force of unfolding and the change of length as the domain unfolds. Unfolding results from breakage of all the non-covalent interactions from the protein secondary and tertiary structure. This uniform stretching is illustrated by the close match between observed unfolding lengths and the prediction simply made from calculating a 0.4-nm extension per amino acid residue. Thioester cross-links were detected by pulling these domains, because the cross-link reduced the distance that the domain could be stretched. With this thioester reporter system in place, the authors then tested the bond's stability in the face of small-molecule nucleophiles (with a free amine). At high concentrations, methylamine and histamine were able to cleave the thioester (to form an amide, Fig. 1) and allow that domain to be stretched fully. It may be that various other amines are present in a physiological context. If there were no control over thioester reactivity, there was a possibility that many of the bacterium's pili would form dead-end complexes with such small molecule nucleophiles, useless for achieving adhesion to the host.

The most striking finding of this study is that after cleaving the thioesters with methylamine, then releasing the tension and washing out the amine, the extension profile of the subsequent force experiment showed that the thioester had reformed. However, if force was maintained on the domains, the thioester did not reform (Fig. 1). This may constitute a mechanism to stabilize anchors but regenerate thioesters from non-productive complexes. It is interesting to contrast Spy0125's direct force sensing with the complicated feedback pathway for mammalian cells to assess their mechanical connection to the extracellular matrix. In mammalian cells there is also force-dependent conformational change, but >150 proteins are associated with a focal adhesion, with regulation through multiple kinases and GTPases (10).

In molecular terms, thioester regeneration by Spy0125 is similar to the first step in the catalytic cycle of a cysteine protease or deubiquitinase, requiring not only a reactive cysteine but also various other surrounding residues to stabilize the transition state for amide bond cleavage. Force acting on the domain could change the local environment around the reactive cysteine and so inhibit this thioester formation. If Spy0125 inactivation were irreversible, this would reflect the cost to the bac-

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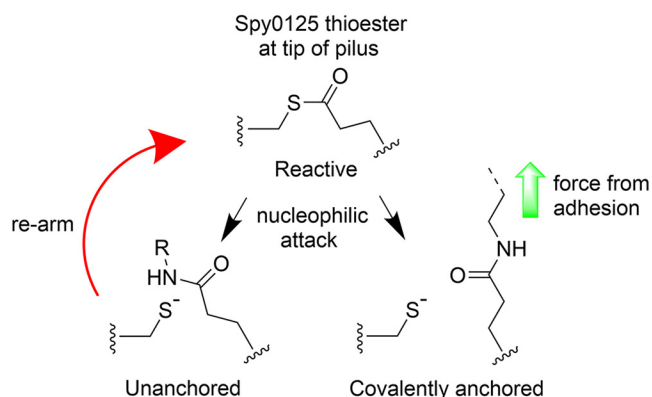


Figure 1. Model of force-dependent covalent adhesion by Spy0125.

terium of synthesizing not just one protein, but potentially the cost of synthesizing the whole pilus.

It seems to be challenging to find the physiological protein ligands for bacterial thioester-containing proteins, even though a number of such proteins have been purified and many more have been identified by sequence homology (6). Breakage of the bond from the bacterial protein to the target ligand in the absence of force may be one reason for this difficulty. Therefore, chemically capping the reactive cysteine or rapid denaturation with urea may help to keep covalent complexes intact. It is also worth investigating what are the most important non-protein molecules reacting with Spy0125 *in vivo*. Drugs to inhibit *S. pyogenes* adhesion by covalently targeting the thioester are an intriguing possibility, but they may have to overcome this proof-reading activity. The surface adhesins SfbI and FbaB from *S. pyogenes* form a stable covalent bond to fibrinogen in the absence of force (6), so there may be diversity in the function of different bacterial thioester-containing domains. It is also still to be determined how big are the forces exerted during *in vivo* infection, although genetic deletions can test the overall importance of specific proteins in different infection models.

Like all of us, proteins sometimes make mistakes. Error correction by enzymes is best known in the context of proof-reading by DNA polymerases. The study by Echelman *et al.* (7) on error correction in adhesion is an important advance in how protein reactivity may be tuned by force, illuminating the sophistication of bacterial surface interactions.

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