THE FIMH ADHESION PROTEIN AS A NANOSCALE MECHANICAL SWITCH

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ABSTRACT

While most adhesive interactions weaken under mechanical force, it has been suggested that some molecular bonds could act as 'catch bonds', and bind more strongly under force. We have shown that the bacterial adhesion protein, FimH, acts as such a catch bond, and have moreover determined at least in part the structural basis for this behavior. Aside from the medical significance of this discovery, there are many technological applications for a nanoscale force sensor that switches on in response to mechanical force.

INTRODUCTION

We have recently discovered that the bacterial adhesion protein called FimH demonstrates the remarkable ability to mediate stronger binding under increased force [1]. This was the first clear demonstration of such a catch bond since the possibility was proposed over a decade ago [2]. While it has long been known that several blood adhesion proteins called selectins demonstrate shear activated adhesion, these proteins appear to work by a completely different mechanism. They have been shown to be 'slip bonds' that weaken under force [3], but movements caused by the fluid velocity increase the bond on-rate enough to increase binding [4].

The discovery that bacterial adhesion can be shear activated is significant to biology and to the study of infectious diseases because it has always been assumed that the flow of bodily fluids works to cleanse human tissue of infectious bacteria. Our research demonstrates that bacteria have evolved a mechanism of overcoming this defense and actually using it for their advantage.

Furthermore, studying how a catch-bond such as FimH is activated by mechanical force can teach us how to reverse engineer nanoscale mechanical switches. This is very important since engineering applications require switching devices that alter the quality or the type of signal, but top-down engineering principles generally fail at the nanoscale. We hope to learn how FimH senses force as well as how it converts force into a change in function.

HOW FIMH WORKS AS A MECHANICAL SWITCH

E. coli and other intestinal bacteria are covered with micron-long appendages called type 1 fimbriae, that present an adhesive protein called FimH at the tip. FimH binds the sugar mannose through its lectin domain, and is firmly attached to the rest of the fimbria through its pilin domain. Mannose is presented on many cell types at the terminus of carbohydrate modifications on various surface proteins, so that FimH binds specifically to a range of host proteins. While all naturally isolated FimH variants bind well to proteins that display a tri-mannose (3Man) moiety, the most abundant variants bind poorly to proteins displaying only mono-mannose (1Man). In our work, we have shown that these poor-binding FimH variants are actually regulated by force, and bind very well to 1Man once force-activated. Discovering how force alters the mannose-FimH structure can teach us how to reverse engineer mechanically sensitive proteins.

Until recently, little was known about how mechanical forces affect the structure and function of the molecules because high resolution experimental methods such as X-ray crystallography can only probe equilibrium structures. We have combined flow chamber experiments that probe FimH adhesive function under force with a novel combination of genetic engineering and molecular simulation techniques to ask how force affects the molecular structure of FimH.

Force Increases Strength of FimH Binding

We have used two types of setups to probe FimH binding under force. Unless noted otherwise, the *E. coli* used in these experiments contained the most common FimH variants that bind 1Man poorly.

In our first experiments [1], we bound the bacteria to tissue culture dishes and then attached red blood cells (RBCs) to the carpet of bacteria and studied the movement of the RBCs under variable shear. We found that the RBCs rolled and even detached under low shear but remained firmly attached with little movement at higher shear. We determined that force and not kinetic effects were behind the shear activation in two ways. First, we were studying cells that were already bound, minimizing any kinetic effects from transport. Second, higher viscosity increased binding strength at lower shear

rates, again pointing to drag force as the cause of the activation, as opposed to transport or on-rate issues. Thus, force increased the binding of the most common, low-1Man-binding, FimH variants to host cells. In contrast, variants of FimH that display increased 1Man binding were shown in these experiments to bind well to RBCs at low or high shear. We have thus demonstrated that FimH binds poorly to 1Man in static conditions, but that binding can be dramatically strengthened by either force or point mutations.

In a second set of experiments [5], we recently bound purified receptors to the surface and floated bacteria through the flow chamber. At low shear (0.1 to 0.5 dynes/cm^2), *E. coli* bound transiently, but rarely stuck, to a surface coated with the 1Man receptor mannose-BSA (manBSA). While more than 28 cells/minute bound per field of view, almost all of these stuck for less than one second before floating freely again, so that less than 0.4 cells/minute accumulated per field of view. This figure of 28 transient adhesions per minute is a lower bound as many transients may have been too short to detect in our system. When the shear was increased to 3.6 dynes/cm^2, 60 *E. coli* accumulated per minute, with no detectable transients. Thus, initial adhesions remained about the same at the low and high shears, but high shear caused a switch from transient to firm adhesion, thus enabling accumulation. This is consistent with the first set of experiments in that shear increases binding strength after binding has occurred.

In contrast, the same *E. coli* bound well to the 3Man receptor RNAseB at both low and high shear with no detectable transients [5]. The accumulation remained the same or dropped with shear as expected for most other adhesive bonds. Thus, force does not further activate binding of FimH to 3Man receptors. Rather, force and complex receptors result in the same type of activation of FimH-mediated binding just as we saw before that some mutations activated FimH.

Force Changes FimH Conformation

We next asked how force could cause a switch from weak to strong binding. There are no experimental methods that give detailed structural information in dynamic conditions, so we used simulations. Steered Molecular Dynamics simulates the effect of an external force on a known molecular structure. We simulated a force on the binding site of the lectin domain and an opposite force on the linker chain to the pilin domain that is anchored to the fimbria. This force caused a conformational change in the lectin domain [1]; the linker chain pulled away from its native position tucked into the loop regions of the binding domain.

However, this conformational change is on the opposite side of the domain from the mannose binding site. Instead, it is in the vicinity of many of the mutations that cause some FimH variants have high 1Man binding. Thus, it is possible that either force or certain mutations can cause structural changes in this region that can in turn cause stronger binding to mannose. This hypothesis was successfully tested by genetically engineering mutations predicted to enhance or inhibit the ability of the linker chain to extend away from its native position, and testing whether they also enhanced or inhibited FimH binding respectively [1].

Conclusion

We have thus demonstrated that FimH binds poorly to 1Man in static conditions, but that binding can be dramatically strengthened by force, point mutations, or the presence of additional mannose residues in the immediate receptor. This suggests that FimH is a regulated molecule with low-binding and high-binding states. While the most common structural variants remain in the low-binding state most of the time, they can be switched to the high binding state by a force-induced conformational change in the linker chain region, or by interactions with the additional mannose residues of 3Man receptors. Other structural variants of FimH with point mutations in the linker chain region are more likely to populate or transition to the high-affinity state prior to activation by force or 3Man.

SIGNIFICANCE

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The catch-bond behavior of FimH may allow bacteria to bind to a surface under shear without binding to high concentrations of soluble inhibitors, as these inhibitors have negligible drag force. It may also allow the bacteria to prevent binding in low-shear regions that bring few new nutrients while still binding firmly in high shear regions. These same mechanisms could allow localization of drug particles to high-shear regions whether to break down artheroschlerotic plaques, or to block bleeding in hemophiliacs.

This research also highlights the importance of studying bacterial infection of biomaterials and implants under shear conditions similar to those presented *in vivo*. It is clear that preventing or inhibiting bacterial adhesion in static conditions does not guarantee that adhesion will be blocked under shear.

Nanotechnology and Reverse Engineering

What we have learned about the mechanism of FimH as a catch-bond can teach how to reverse engineer mechanical sensors and switches. In particular, we saw that a force-induced conformational change in one region can propagate to another part of the protein to change its function, in this case mannose binding. It is already well understood in biochemistry that the equilibrium between low- and high-activity states of an 'allosteric' protein can be regulated by the binding of a soluble molecule distant from the active site. We propose that FimH is an allosteric protein regulated by force. This is a uniquely nano-scale mechanism, utilizing the principles of thermodynamics rather than macro-scale mechanics. This mechanism can in principle be used to couple force to any chemical process for which we already know of an allosterically regulated protein. Thus FimH suggests a method to reverse engineer a broad spectrum of nanoscale chemo-mechanical switches. In the shorter term, FimH could be used directly as a mechanical coupler with the unusual property of only binding under force.

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