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Catch-Bond Behavior of Bacteria Binding by Slip Bonds

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ABSTRACT It is shown that multipili-adhering bacteria expressing helix-like pili binding by slip bonds can show catch-bond behavior. When exposed to an external force, such bacteria can mediate adhesion to their hosts by either of two limiting means: sequential or simultaneous pili force exposure (referring to when the pili mediate force in a sequential or simultaneous manner, respectively). As the force is increased, the pili can transition from sequential to simultaneous pili force exposure. Since the latter mode of adhesion gives rise to a significantly longer bacterial adhesion lifetime than the former, this results in a prolongation of the lifetime, which shows up as a catch-bond behavior. The properties and conditions of this effect were theoretically investigated and assessed in some detail for dual-pili-adhering bacteria, by both analytical means and simulations. The results indicate that the adhesion lifetime of such bacteria can be prolonged by more than an order of magnitude. This implies that the adhesion properties of multibinding systems cannot be directly conveyed to the individual adhesion-receptor bonds.

INTRODUCTION

Specific noncovalent bonds between biologically active molecules, such as adhesins and their receptors, traditionally have been assumed to follow the kinetic theory of Kramers (1) and Bell (2), and are referred to as slip bonds. This implies, among other things, that their lifetime decreases with force. However, from time to time, evidence has appeared of systems in which the bond lifetime increases with force. The existence of such systems, referred to as catch bonds, was first proposed by Dembo et al. (3) some 20 years ago. Since then, catch bonds have been confirmed experimentally for P-selectin (4–7) and L-selectin (8) binding to P-selectin glycoprotein ligand-1, for actin binding to myosin (9), for fibronectin binding to integrin (10), and for the specific adhesion of FimH expressed by *Escherichia coli* type 1 pili to its receptor monomannose (11–13). The methods used to demonstrate and assess the catch-bond phenomenon vary from measurements on a single-molecule level to stick-and-roll measurements acquired in studies investigating the adhesion properties of bacteria that bind by multiple bonds and are exposed to skew forces.

The catch-bond phenomenon is still somewhat of a mystery, and no single conclusive explanation has yet been presented (14). Instead, investigators have proposed a variety of possible causes of the catch-bond phenomenon. One is based on the assumption that the adhering molecules can bind to the host with two or more conformational states between which transitions can take place (4,15). Another assumes that the molecules possess more than one possible escape path, and the paths have dissimilar force dependencies (16,17). A third explanation is that the proteins involved undergo a structural conformational change, which results in a firmer attachment (18,19).

One of the systems in which the catch-bond phenomenon has been observed—the binding of type 1 pili from *E. coli* to monomannose (11–13,20,21)—differs from the others in one important aspect: the FimH adhesin is not expressed directly on the surface of the cell, but rather is supported by a helix-like adhesion organelle, referred to as a pilus. The force response of helix-like pili, which has been scrutinized in detail on an individual pilus level for various types of pili expressed by *E. coli* (predominantly P pili (22–25), type 1 (26), and S pili (27)), has been found to be nonlinear and to give rise to a complex biomechanical behavior (28,29). The knowledge obtained from such studies formed the basis for recent simulations of the adhesion properties of multipili-adhering bacteria exposed to external forces (30). It was found that the adhesion lifetime of multipili-binding bacterial systems depends not only on the adhesin-receptor (AR) bond, but also to a large degree on the internal biomechanical properties of the individual pili. In particular, it was concluded that the cooperativity of the pili plays a crucial role in the ability of multipili-adhering bacteria to stay attached to host tissue in the presence of an external force (30).

On the basis of this pili cooperativity, we present in this work an alternative explanation for the catch-bond behavior of bacteria adhering to hosts by multiple helix-like pili. We propose that it is possible for a multipili-adhering bacterium to display an adhesion lifetime that increases with the force to which it is exposed, although the AR bonds by which the pili adhere to the host are of the slip-bond type. Since the signature of a catch bond (when single-molecule adhesion is studied) is an adhesion lifetime that increases with force, the corresponding response of a multipili-adhering bacterium will in this work be referred to as bacterial catch-bond behavior (or simply catch-bond behavior, for short). We want to emphasize that such a behavior does not imply that any of the pili's AR bonds need to express catch-bond behavior; it is the bacterium as a multipili adhesion

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system that expresses an increased adhesion lifetime with force.

To make the description and analysis of the phenomenon manageable and comprehensible, this work is restricted to bacteria that bind by two helix-like pili, henceforth referred to as dual-pili-adhering bacteria. Bacteria that bind by more pili will be dealt with elsewhere. Here we present a model that considers dual-pili-adhering bacteria whose adhesins are of the slip-bond type. It is shown that such bacteria can, under a certain range of conditions, exhibit a catch-bond behavior. Building on our previous investigation of the response of multipili-adhering bacteria exposed to external forces (30), we first present a simplified theoretical description (by analytical means) of the catch-bond phenomenon in a dual-pili-adhering system. This description both demonstrates the fundamental mechanism of the phenomenon and provides the conditions under which it can take place. To better understand this phenomenon and assess it quantitatively, we simulated it in more detail using a Monte Carlo approach. The simulations indicate clearly that the cooperativity of pili can cause dual-pili-adhering bacteria expressing helix-like pili binding by slip bonds to exhibit catch-bond behavior.

THEORY

Bond kinetics

Single-bond kinetics

The kinetics of an individual bond can be both envisioned and understood by means of an energy landscape, as is schematically illustrated in Fig. 1. State A represents a closed conformation (i.e., when the bond is "on" or "closed"), whereas state B corresponds to an open conformation (i.e., when the bond is "off" or "open"). State T is the transition state through which the bond has to pass before it can either open or close (i.e., to make the transformation from state A to B or vice versa, respectively). In the case of a single-state bond, there is no open state B. This case is depicted by the dashed line in Fig. 1.

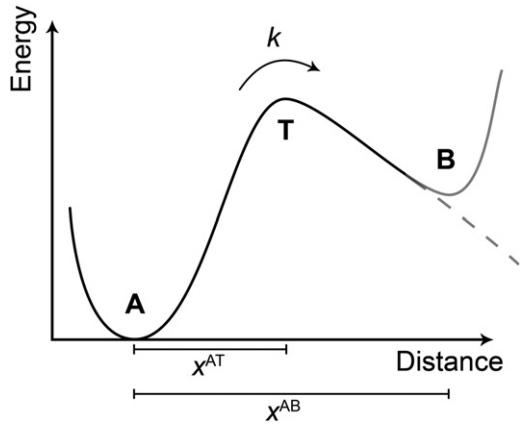

FIGURE 1 Schematic illustration of a two-state energy landscape. The transition rate, k, for a bond in state A to open to state B is restricted by the transition barrier T. In systems in which the two states represent dissimilar conformational states of a bond, state B constitutes a local minimum (*solid line*). In the case of a single-state bond, such as an AR slip bond, there is no local minimum at B (*dashed line*). It is assumed that such an open bond will not close, i.e., the system will not make any transition from state B to state A.

The opening rate of a slip bond exposed to a force, $k(F)$, also referred to as the off-rate in the literature, is here assumed to follow the conventional theory given by Bell (2), which implies that it is given by a product of a thermal opening rate k^{th} and an Arrhenius factor taking into account the influence of the force, F, and comprising the bond length, x^{AT}, the Boltzmann constant, k_{B}, and the temperature, T:

$$k(F) = k^{\mathrm{th}} e^{\frac{F x^{\mathrm{AT}}}{k_{\mathrm{B}} T}} \tag{1}$$

A single bond exposed to a time-dependent force, $F(t)$, which thus experiences a time-dependent opening rate, $k[F(t)]$, has a lifetime, $\langle t \rangle_1$, given by

$$\langle t \rangle_1 = \int_0^\infty k[F(t)] t e^{-\int_0^t k[F(t')]dt'} dt \tag{2}$$

For a constant force, the lifetime simplifies to the inverse of the opening rate, which for an AR bond of the slip-bond type can be written as

$$\langle t(F) \rangle_1 = k^{-1}(F) = \frac{1}{k_{\mathrm{AR}}^{\mathrm{th}}} e^{-\frac{F}{F_{\mathrm{AR}}}} \tag{3}$$

where F_{AR} is referred to as the characteristic force for the AR bond and is defined as $k_{\mathrm{B}}T/x_{\mathrm{AR}}^{\mathrm{AT}}$. This shows that the lifetime of a single slip bond is strongly affected by the force to which it is exposed; an increase in force by one order of magnitude, from F_{AR} to $10F_{\mathrm{AR}}$, shortens the lifetime by almost four orders of magnitude.

Multibond kinetics—cooperativity

It was shown by Björnham and Axner (30) that under a few reasonable conditions, predominantly that the system is exposed to such a force that the opening rate for an individual bond not exposed to force can be neglected with respect to that of a force-exposed bond, the lifetime of a bacterial system adhering by N bonds in which the bonds are exposed to a force in a sequential manner, i.e., one bond is exposed to the entire force at a time (when the bond exposed to force ruptures, another bond takes up the entire force, etc.), henceforth referred to as sequential force exposure, can be written as

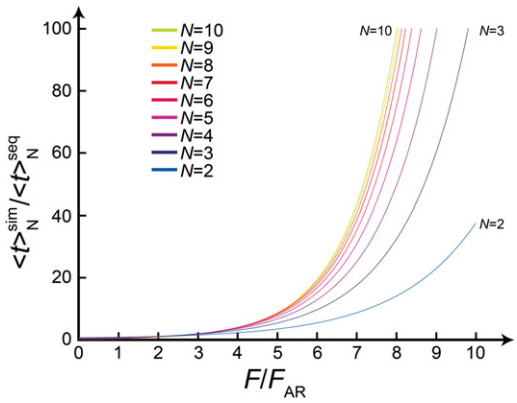

FIGURE 2 (Color online) The importance of pili cooperativity is illustrated by the ratio of the lifetimes of a system with N bonds with simultaneous and sequential pili force exposure, respectively, as a function of force, expressed in units of the characteristic force. The nine curves represent (from the *bottom* to the *top*) systems that bind by two to bonds.

$$\langle t(F)\rangle_{\mathrm{N}}^{\mathrm{seq}} = N\langle t(F)\rangle_1 \tag{4}$$

Since the bond-opening rate increases exponentially with force (according to Eq. 1), a system that binds by several bonds will experience a substantially longer lifetime if the force is distributed among some or all of the bonds (so no AR bond will experience an excessive force) than if the bonds are exposed to force in a sequential manner. For a system with full cooperativity, i.e., in which all bonds are simultaneously exposed to force and share it equally (referred to as simultaneous force exposure), the adhesion lifetime can instead be written as

$$\langle t(F)\rangle_{\mathrm{N}}^{\mathrm{sim}} = \frac{1}{k_{\mathrm{AR}}^{\mathrm{th}}}\sum_{n=1}^{N}\frac{1}{n}e^{-\frac{F}{nF_{\mathrm{AR}}}} \tag{5}$$

This implies that a system adhering by N bonds with simultaneous force exposure will experience a longer lifetime than a system with sequential force exposure by a factor of

$$\frac{\langle t(F)\rangle_{\mathrm{N}}^{\mathrm{sim}}}{\langle t(F)\rangle_{\mathrm{N}}^{\mathrm{seq}}} = \frac{\sum_{n=1}^{N}\frac{1}{n}e^{-\frac{F}{nF_{\mathrm{AR}}}}}{Ne^{-\frac{F}{F_{\mathrm{AR}}}}} \tag{6}$$

This entity, which is a measure of the cooperativity of pili, is illustrated in Fig. 2 for systems adhering by 2–10 bonds exposed to an external force up to $10F_{\mathrm{AR}}$. The figure shows that pili cooperativity is important for the adhesion of multi-bond-adhering bacterial systems exposed to external forces. The adhesion lifetime is significantly longer in systems in which several bonds share the force compared to those in which the bonds mediate the force in a sequential manner, particularly when the system is exposed to forces above a few times the characteristic force, F_{AR}.

For bacteria that express their adhesins directly on the cell wall, it is unlikely that an external force can be shared reasonably equally among a multitude of bonds. This is because an external force acting on a bacterium adhering to a host cell often acts as a skew force, which gives rise to a strongly uneven force distribution among the various adhesins. On the other hand, for bacteria that express their adhesins on the tip of extendable attachment organelles, and in particular those that have a helix-like structure that can be extensively elongated when exposed to stress, the situation is generally the opposite: the nonlinear elongation behavior of organelles makes it possible for an external force to be distributed among a large number of pili, possibly even fairly equally. This implies that bacteria adhering by expandable pili will experience a substantially longer adhesion lifetime than those that lack pili (30).

Helix-like pili

Structure and biomechanical properties

Helix-like pili are constructed by hundreds or thousands of identical subunits that are linearly connected to each other by so-called head-to-tail (HT) bonds, comprising the backbone of the pili. This structure is, in turn, coiled into a helix-like quaternary structure by means of layer-to-layer (LL) bonds that connect nonconsecutive subunits to each other, predominantly the subunits n and $n+3$, as is illustrated in the left part of Fig. 3 *A*. As is shown in the right part of the same panel, under exposure to a sufficiently large external force, these LL bonds will open in a sequential manner, resulting in an uncoiling (also referred to as "unfolding" in the literature) of the helix-like quaternary structure of the pili into a linear conformation (22,24,28,29).

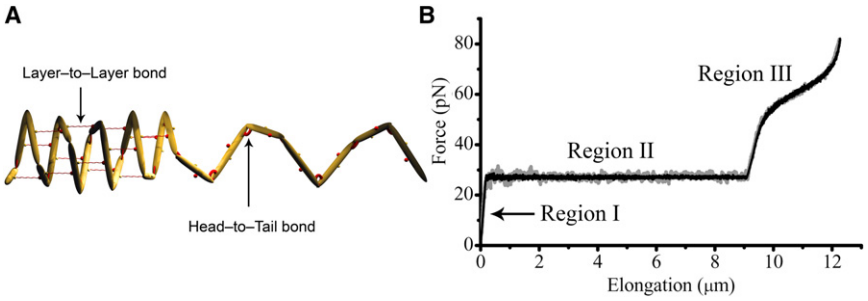

FIGURE 3 (Color online) (*A*) A schematic illustration of the geometric conformation of a helix-like pilus. The left part represents a pilus in its helix-like conformation, whereas the right part depicts the pilus when it has been uncoiled. (*B*) A force response curve (*gray*) of a P pilus from an elongation measurement obtained with optical tweezers. The black curve represents a fit obtained using a previously developed simulation program. The extended region II corresponds to the uncoiling of the helix-like structure.

Since this uncoiling is sequential, the pili elongate under a constant force, which is referred to as the uncoiling (unfolding) force and is represented by region II in a force-versus-elongation plot such as that shown in Fig. 3 *B*. For elongations at low velocities, below the corner velocity, the uncoiling takes place at a velocity-independent force referred to as the steady-state uncoiling (unfolding) force. For high elongation velocities, the uncoiling force increases (logarithmically) with the velocity. This implies that for forces above the steady-state uncoiling force, the elongation takes place at a velocity given by the force (with an uncoiling velocity that depends exponentially on the applied force) (23). When the entire helix-like structure has been linearized, the HT bonds will, during continued pili elongation, rapidly take up a larger force whereby they can be subjected to further conformational changes. This gives rise to a wave-shaped force-versus-elongation dependence, referred to as region III (22,24).

It was recently suggested that this nonlinear elongation behavior is of the highest importance for the cooperativity of pili, which in turn affects the adhesion lifetime of bacteria (30). Of special significance is the force plateau in region II, which for P pili expressed by *E. coli* elongating under steady-state conditions takes place at 28 pN, because it leads to a significant elongation of the pili, often by a factor of ~5.

Finally, the adhesin, which binds specifically to its receptor by an AR bond, is located at the distal end of the pilus. This implies that the AR bond takes up the same force as the pilus.

Theoretical description and simulations

Previous assessments of the biomechanical properties of individual pili have made it possible to both model and simulate the force-versus-elongation properties of pili with good agreement with experimental findings (22–29). As an example, the gray curve in Fig. 3 *B* displays the nonlinear force-versus-elongation response from a P pilus expressed by *E. coli*, whereas the black curve represents a fit using a simulation program described in Björnham et al. (31). As can be seen from the figure, there is an excellent agreement between the simulated behavior and the measured data. This shows that the previously developed theoretical description (24) and simulation procedure can mimic the force-versus-elongation response of individual P pili well (31).

Adhesion lifetime of dual-pili-adhering bacteria exposed to force

To date, no systematic experimental study of the adhesion properties of multipili-adhering bacteria exposed to external forces, with the number of force-mediating pili known (thus excluding the rather complex stick-and-roll investigations), has been performed; however, the adhesion lifetime of such bacteria was recently scrutinized in some detail by Björnham and Axner using Monte Carlo simulations (30). It was concluded that the cooperativity of pili plays a crucial role in the adhesion lifetime of such bacteria, and that it is largest for systems in which all force-exposed pili are simultaneously elongated in region II. If one pilus halts in region I, it will take a smaller force than the others. Since its AR bond will then experience a lower opening rate than the others, the pilus will not fully contribute to the cooperativity of the system. Similarly, if a pilus enters region III, it will take a larger force than the others, whereby the opening rate of its AR bond will be increased and the cooperativity (and thus the adhesion lifetime of the system) decreased. Since the opening rate of the AR bond of a pilus that has halted in region I is low, and it is expected that many types of multipili-adhering systems will detach before any pilus reaches region III (30), the analytical analysis of this work will, for simplicity, be restricted to the case in which the force-exposed pili are elongated in region II. In contrast, the simulations, which are presented further below, incorporate all properties of the pili elongation, including the elongations in regions I and III.

Moreover, when an elongated helix-like pilus detaches, it will quickly recoil. The adhesin will thus be rapidly separated from its receptor, which prevents rebinding. This implies that rebinding of detached helix-like pili can be assumed to play a minor role in the bacterial detachment process. Hence, when the biomechanical behavior of pili and the adhesion lifetime are modeled, pili rebinding can be neglected. For the other bonds in the system, i.e., the LL and HT bonds, both the opening and the closure rates need to be considered, since they can open and close a multitude of times before the adhesin detaches from its receptor.

Expected uncoiling length of a single pilus

It was previously concluded (30) that the expected uncoiling length of a pilus elongating in region II, $< L_{\mathrm{II}}(F) >$, plays an important role in the adhesion lifetime of multipili-adhering bacterial systems. Under the condition that the pilus is exposed to a constant force, this entity is given by

$$< L_{\mathrm{II}}(F) > = \frac{x_{\mathrm{LL}}^{\mathrm{AB}}}{\eta_{\mathrm{k}}} e^{(1-\eta_{\mathrm{x}})\frac{F}{F_{\mathrm{LL}}}} \qquad (7)$$

where η_{k} and η_{x} are defined as the ratio of the thermal bond opening rates and the ratio of the bond lengths for the AR and LL bonds, i.e., $k_{\mathrm{AR}}^{\mathrm{th}}/k_{\mathrm{LL}}^{\mathrm{th}}$ and $x_{\mathrm{AR}}^{\mathrm{AT}}/x_{\mathrm{LL}}^{\mathrm{AT}}$, respectively. Equation 7 shows the important fact that the expected uncoiling length can either increase or decrease as a function of force, depending on the ratio of the two bond lengths, i.e., η_{x}. For systems in which $\eta_{\mathrm{x}} < 1$, i.e., for which the bond length of the AR bond is shorter than that of the LL bond, the expected uncoiling length will increase with force, whereas it will decrease for systems in which $\eta_{\mathrm{x}} > 1$.

Qualitative description of the bacterial catch-bond behavior of a dual-pili-adhering system

When a bacterium binds by more than one pilus, the various pili will, in general, become exposed to force at dissimilar bacterium-to-host distances, henceforth referred to as different force onset positions, s_i. If the expected uncoiling length in a dual-pili system is shorter than the difference in force onset positions, Δs_{12}, given by $|s_1 - s_2|$, the cooperativity will be low (most of the time, the adhesion is mediated by only one pilus at a time, i.e., the system is experiencing a sequential pili force exposure), and thus the lifetime will become short. For systems with $\eta_x < 1$, as the force is increased, the expected uncoiling length will do the same, which will lead to an increased probability that the force will be shared between the two pili (i.e., simultaneous pili force exposure), which in turn will result in an increased cooperativity and thus a prolonged adhesion lifetime. This indicates that a dual-pili-adhering system can exhibit a short lifetime for low forces (when it mediates adhesion by sequential pili force exposure, for which the pili cooperativity is low) but a long lifetime for high forces (when it mediates adhesion by simultaneous pili force exposure, for which the pili cooperativity is high), which is nothing but the signature of a catch bond. The catch-bond behavior can then appear in the transition between these two limiting cases. The entity η_x is therefore of high importance for the cooperativity of pili and hence the ability of a multipili-adhering bacterial system adhering by slip bonds to exhibit catch-bond behavior.

Quantitative assessment of the bacterial catch-bond behavior of a dual-pili-adhering system

It is possible to assess the conditions under which a dual-pili-adhering system will display catch-bond behavior more rigorously, in the following way: As previously shown (30), under some general conditions (primarily under a constant force exposure and for an equal distribution of the force between the two pili, whenever both take up force), the expected lifetime of a dual-pili-adhering system elongated in region II can be written as

$$\langle t(F)\rangle_2 = \frac{2}{k(F)} + \left[\frac{1}{2k(F/2)} - \frac{1}{k(F)}\right] P_{AR}(F, t_d) \quad (8)$$

where $P_{AR}(F, t_d)$ is the probability that the first pilus that mediates force is still attached at the time when the second one becomes exposed to force, t_d, given by

$$P_{AR}(F, t_d) = e^{-k(F)t_d(F)} \quad (9)$$

where $t_d(F)$ is given by the ratio of the difference in force onset positions for the two pili and the elongation velocity, $\dot{L}(F)$, i.e., $\Delta s_{12}/\dot{L}(F)$, where, in turn, $\dot{L}(F)$ is the elongation velocity of a single pilus exposed to force.

The bacterial catch-bond behavior is characterized by an increasing lifetime with force, i.e., a positive first-order derivative of the lifetime with respect to force, $d\langle t(F)\rangle_2/dF > 0$. Using Eq. 8, this derivative can be written as

$$\frac{d\langle t(F)\rangle_2}{dF} \approx [2k(F)t_d(F)(1-\eta_x) - \eta_x]\frac{P_{AR}(F, t_d)}{4F_{LL}k(F/2)} - \frac{2\eta_x}{k(F)F_{LL}} \quad (10)$$

where the assumption that $k(F) \gg k(F/2)$ has been used. This implies that the condition for a bacterial catch-bond behavior can be written as

$$K_1(F)K_2(F) > 1 \quad (11)$$

where

$$\begin{aligned} K_1(F) &\equiv 2k(F)t_d(F)^{\frac{(1-\eta_x)}{\eta_x}} - 1 \\ &= 2\eta_k\overline{\Delta}s_{12}(1-\eta_x)e^{-\frac{(1-\eta_x)}{\eta_x}\frac{F}{F_{AR}}} - 1 \end{aligned} \quad (12)$$

and

$$\begin{aligned} K_2(F) &\equiv \frac{P_{AR}(F, t_d)}{8}\frac{k(F)}{k(F/2)}, \\ &= \frac{1}{8}e^{-k(F)t_d(F)}e^{\frac{F}{2F_{AR}}} \end{aligned} \quad (13)$$

where we have introduced $\overline{\Delta}s_{12}$, referred to as the normalized difference in force onset position, to denote the ratio of the difference in force onset positions for the two pili and the bond length of the AR bond, i.e., $\Delta s_{12}/x_{AR}^{AT}$, respectively. Since $K_2(F)$ is always larger than zero, this implies that a bacterial catch-bond behavior requires

$$K_1(F) > \frac{1}{K_2(F)} > 0 \quad (14)$$

This equation provides both a lower and an upper limit for the force interval in which catch-bond behavior from a dual-pili-adhering system can take place.

As can be seen by inspection, for systems with $\eta_x < 1$, K_1 decreases with force. Moreover, since both the probability that the first pilus is still attached at the time when the second pilus becomes exposed to force, $P_{AR}(F, t_d)$, and the ratio of the opening rates of the AR bond of a pilus exposed to the entire or half of the force, $k(F)/k(F/2)$, increase with force, K_2 does the same. For low forces, K_2 is too small for Eq. 11 to be fulfilled, whereas for high forces, K_1 becomes too small (and even negative) to fulfill Eq. 14. It is possible, though, for Eq. 14 to be fulfilled for intermediate forces. In addition, Eq. 12 provides a lower limit of η_k. For K_1 to be larger than $1/K_2$ when K_2 starts to take appreciable values (i.e., in practice $K_1 > 0$), η_k cannot be too small; it has to be significantly larger than $1/\overline{\Delta}s_{12}$, which for helix-like pili typically is $\sim 10^{-3}$.

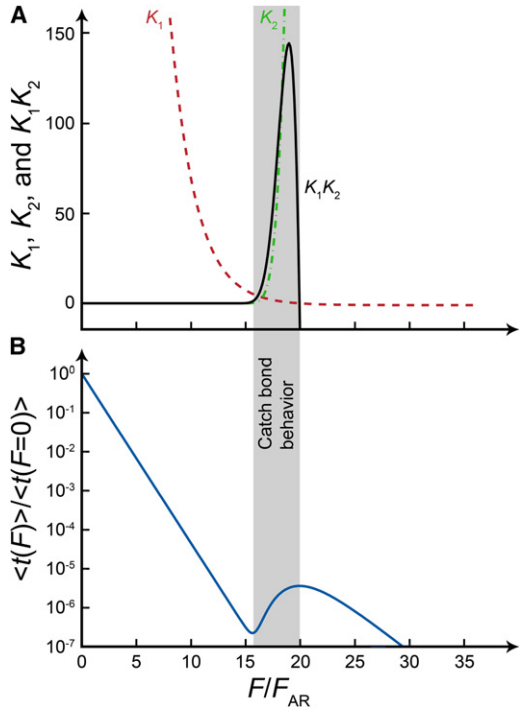

FIGURE 4 (Color online) A dual-pili system exposed to force. Panel *A* shows the behavior of K_1 (*dashed light gray*) and K_2 (*semi-dashed dark gray*), as well as their product, K_1K_2 (*solid black*), whereas panel *B* displays the normalized lifetime of the system as a function of normalized force. The shaded band marks the force interval in which the catch-bond behavior takes place, which for panel *A* is given by the conditions of Eq. 11.

Fig. 4 provides an example of bacterial catch-bond behavior of a dual-pili-adhering bacterial system in which all bonds are slip bonds. Panel *A* illustrates the behavior of K_1, K_2, and K_1K_2, whereas panel *B* displays the adhesion lifetime of the system, $\langle t \rangle_2$, as a function of force for a system for which $\eta_x = 0.7$, $\eta_k = 10$, and $\overline{\Delta}s_{12} = 6000$. Fig. 4 *A* shows that, as F is increased, K_2 increases monotonically from a low value (~0), whereas K_1 decreases monotonically from a high value. Since K_2 is strongly nonlinear with force, it will, for the particular values of η_x and η_k considered, exceed $1/K_1$ for a force of ~16 F_{AR}. On the other hand, K_1 will become smaller than $1/K_2$ (as well as 0) for a force of 20 F_{AR}. Hence, the product K_1K_2 takes in this case a value above unity in the force interval $16\ F_{AR} < F < 20\ F_{AR}$, indicating that the system should display a catch-bond behavior in this region (shaded in the figure for clarity). Fig. 4 *B* shows that the lifetime indeed increases with force within this force interval, verifying the analytical analysis of the conditions for a bacterial catch-bond behavior given above, as well as the existence of a catch-bond behavior of a dual-pili-adhering system adhering by slip bonds.

Undoubtedly, the most important factors in the bacterial catch-bond behavior are the ratios of the bond lengths for the AR and LL bonds, η_x, and the thermal bond opening rates, η_k. First of all, they determine in which force interval the catch-bond behavior can take place. It can be concluded from the analysis above that low values of both η_x and η_k, which take place when the bond length of the AR bond is significantly smaller than that of the LL bond, i.e., $x_{AR}^{AT} < x_{LL}^{AT}$, and when the thermal uncoiling rate of the AR bond is significantly smaller than the thermal opening rate of the LL bond, i.e., $k_{AR}^{th} < k_{LL}^{th}$, respectively, imply that bacterial catch-bond behavior takes place at low forces, whereas high values result in the opposite.

These two ratios also determine how large the increase in bacterial adhesion lifetime will be (hereafter referred to as the catch-bond effect). Since the lifetime of a single pilus depends exponentially on the force, i.e., as $\exp(-F/F_{AR})$ (according to Eq. 3), whereas that of a dual-pili-adhering system has a weaker force dependence, i.e., as $\exp(-F/2F_{AR})$ (according to Eq. 5), the transition from sequential to simultaneous force-mediated adhesion prolongs the lifetime more for high than for low forces (which also can be seen from Eq. 6). This means that high values of η_x and η_k (although still with the requirement that $\eta_x < 1$) will, in general, give rise to a stronger catch-bond effect than low values.

The extent to which the bacterial adhesion lifetime can be prolonged by the catch-bond behavior for a given set of η_x and η_k could, in principle, be assessed by Eq. 8. However, since this expression is derived under a few assumptions, it does not include all possible elongation conditions. It is primarily restricted to the case in which the pili elongate in region II, when they are exposed to a constant force, and when this force is equally distributed among the two pili. It is thereby valid only if the force is above twice the steady-state uncoiling force. If it is below this, one of the two pili will halt in region I and the other most probably will reside in region II, giving rise to an uneven force distribution among the two pili. Moreover, for long bacterial adhesion lifetimes, one pilus is likely to enter region III before the bacterium detaches. This also gives rise to a situation in which the distribution of force among the two pili is uneven. Although it is possible to conclude that the cooperativity (and thus the catch-bond effect) is decreased in both these cases, the conditions for Eq. 8 are violated, and therefore it cannot be used to quantitatively assess the cooperativity and size of the catch-bond effect. On the other hand, these can be conveniently assessed by simulations based on expressions for the opening and closure rate of the LL bonds as well as the opening rate of the AR bond (according to Eq. 1), since such investigations do not put any restrictions on the force distribution or the elongation regions. This justifies a more thorough investigation by simulations of the appearance of the catch-bond behavior from dual-pili-adhering bacteria binding solely by slip bonds.

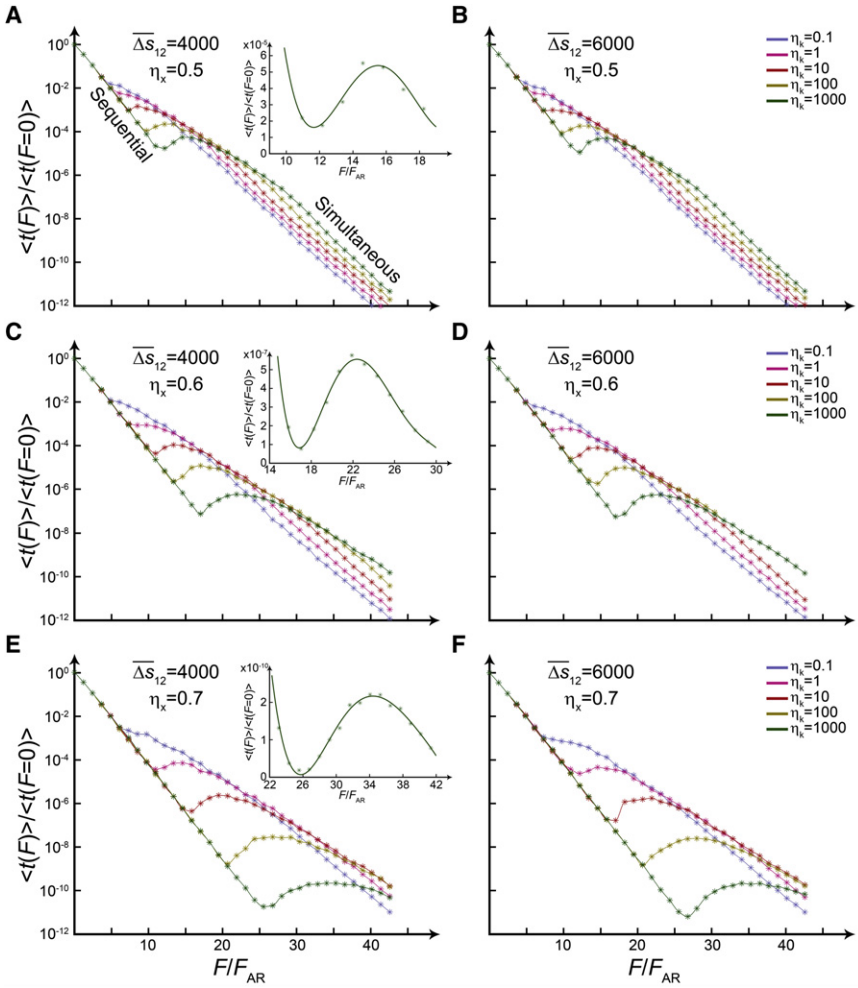

FIGURE 5 (Color online) The normalized lifetime of dual-pili-adhering systems as a function of normalized force (in terms of the characteristic force, F_{AR}). The various panels represent the following conditions: (*A* and *B*) $\eta_x = 0.5$; (*C* and *D*) $\eta_x = 0.6$; (*E* and *F*) $\eta_x = 0.7$; (*A*, *C*, and *E*) $\overline{\Delta s}_{12} = 4000$; and (*B*, *D*, and *F*) $\overline{\Delta s}_{12} = 6000$. The various curves in each panel represent η_k values of 0.1, 1, 10, 100, and 1000. The insets show the catch-bond effect with a linear timescale for $\eta_k = 1000$. The limiting type of pili force exposure that is explicitly indicated in panel *A* is also valid in the other panels.

Simulation procedures

The simulation procedures are described in the Supporting Material. The parameter values used in the simulations are summarized in Table S1.

RESULTS AND DISCUSSION

As alluded to above, a bacterial catch-bond behavior might occur in dual-pili-adhering systems in which the expected uncoiling length increases with force, which it does for systems in which the bond length of the AR bond is shorter than that of the LL bond, i.e., $\eta_x < 1$. In addition, when exposed to low forces, the systems need to have an expected uncoiling length, $< L_{II}(F) >$, that is short in comparison to the difference in force onset positions of the pili, Δs_{12}. Under such conditions, the systems will experience primarily a sequential pili force exposure, which implies that their lifetime will be short. As the force is increased, the expected uncoiling length will increase. A catch-bond behavior can then take place if the expected uncoiling length starts to rival (and exceed) the difference in the force onset positions of the pili. In this case, both pili will start to mediate force, which implies that the bacterial systems have transit into systems with simultaneous pili force exposure, which in turn leads to a prolongation of the lifetime.

This phenomenon is illustrated in Fig. 5 for a few sets of parameter values. The various panels show the ratio of the lifetimes of a few dual-pili-adhering bacterial systems in the presence and absence of force, respectively, i.e., $\langle t(F)\rangle/\langle t(F=0)\rangle$, as a function of the applied force for a variety of conditions (given in the figure caption).

The leftmost parts of the curves in all panels show the same behavior, a lifetime that decreases exponentially with force, irrespective of the various parameter values. This is because under these conditions (when the expected elongation length of a pilus is shorter than the difference in force onset positions, Δs_{12}), the systems mediate their adhesion by sequential pili force exposure. The lifetimes are given by the ratio of Eq. 4 in the presence and absence of force, i.e., $\langle t(F)\rangle_2^{seq}/\langle t(F=0)\rangle_2^{seq}$, which is equal to $\exp(-F/F_{AR})$ and gives rise to a straight line in a lin-log plot. This behavior is in agreement with previous experimental results, such as those of Kong et al. (10).

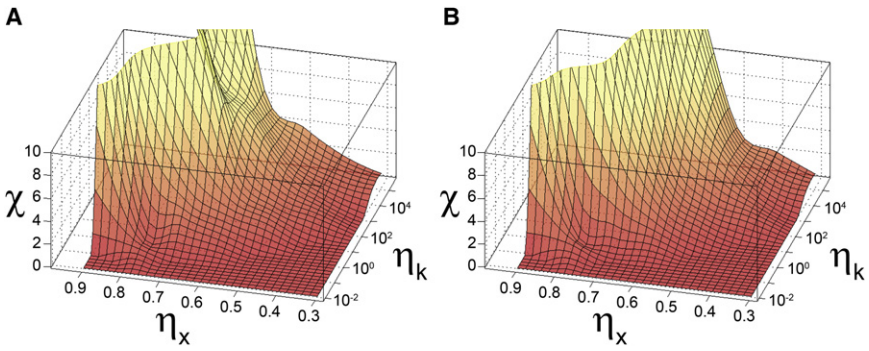

FIGURE 6 (Color online) The relative catch-bond effect as a function of η_x and η_k, ranging from 0.3 to 0.9, and 10^{-2} to 10^{5}, respectively. Panels *A* and *B* represent normalized differences in force onset position, $\overline{\Delta}s_{12}$, of 4000 and 6000, respectively.

For high forces, the various curves show a similar type of behavior, i.e., a lifetime that decreases exponentially with force. In this case, however, the systems are mediating their adhesion mainly by simultaneous pili force exposure (originating from an expected elongation length that is longer than the difference in force onset positions). The lifetimes are in this case roughly given by a ratio similar to that of Eq. 5 in the presence and Eq. 4 in the absence of force (although the former is strictly valid only in the case of equal force distribution).

As alluded to above, a bacterial catch-bond behavior can take place in the transition between these two limiting situations. It shows up as a positive first-order derivative of the lifetime with respect to force (i.e., whenever there is a local minimum followed by a local maximum in the lifetime-versus-force plot) and it appears whenever the expected elongation length, $< L_{II}(F) >$, starts to be comparable to the difference in force onset positions. It can be seen in the figure that the catch-bond behavior can take place under a variety of conditions. For an η_x value of 0.5 (Fig. 5, *A* and *B*), it takes place for $\eta_k \geq 100$, whereas for $\eta_x = 0.7$ (panels *E* and *F*), it appears for $\eta_k \geq 1$. In addition, the upper and lower force limits for the bacterial catch-bond behavior depend to a large extent on η_k; for the case of $\eta_x = 0.7$ it ranges from 11 F_{AR} to 15 F_{AR} for $\eta_k = 1$, whereas it extends from 25 F_{AR} to 36 F_{AR} for $\eta_k = 1000$. This shows that for lower values of η_x, the limits appear at lower force values. Moreover, the simulations indicate that there are relatively small differences in the catch-bond behavior between systems with a normalized difference in force onset positions of 4000 and 6000, respectively.

The insets show the catch-bond behavior in a zoomed perspective and in a linear scale. These insets illustrate that the catch-bond behavior can prolong the adhesion lifetime of dual-pili-adhering bacteria substantially, by factors of 3, 7, and 12, for systems for which η_x is equal to 0.5, 0.6, and 0.7, respectively.

Under the assumption that the thermal lifetime of the AR bond is on the order of 10^3 s, Fig. 5 shows that the catch-bond behavior increases the lifetime of a dual-pili-adhering bacterial system with $\eta_x = 0.5$ roughly from some tens of milliseconds to nearly 100 ms for $\eta_k = 1000$, whereas the corresponding change in lifetime for a $\eta_x = 0.6$ system increases from tenths of milliseconds to milliseconds. These predicted lifetimes are shorter than the typical force exposure time of bacterial systems expressing helix-like pili, e.g., *E. coli* in the urinary tract. However, as is further discussed below, in an in vivo situation, bacteria are able to bind with more than two pili, which can result in a prolongation of the lifetimes beyond what can be obtained in dual-pili-adhering systems.

To quantify and assess the conditions for the catch-bond behavior, we found it convenient to introduce an entity that is a measure of the relative increase in bacterial adhesion lifetime, henceforth referred to as the relative catch-bond effect, χ, defined as $\max\{\langle t(F_j)\rangle - \langle t(F_i)\rangle\}/\langle t(F_i)\rangle$, where F_i and F_j are the forces corresponding to the local minimum and maximum of the lifetime-versus-force curves, respectively (where thus $F_j > F_i$). For the case in which there is no catch-bond behavior, it is defined as zero. This entity thus describes how much the lifetime can be prolonged (on a relative scale) in a dual-pili-adhering system by the catch-bond behavior. It was found that this entity can take significant values, which indicates the true importance of pili cooperativity of multipili-adhering systems. Fig. 6, *A* and *B*, show the relative catch-bond effect of dual-pili-adhering systems as a function of the ratios of the bond lengths and the thermal bond opening rates for the AR and LL bonds, i.e., η_x and η_k, for two different normalized differences in force onset position (with the χ axes restricted to 10).

Fig. 6 shows that bacterial catch-bond behavior takes place for all η_x values investigated, ranging from 0.3 to 0.9. For the highest η_x values ($\geq$0.8), the effect appears for a large range of η_k values ($>10^{-1}$), whereas for the lowest η_x values (~0.3), it only takes place for η_k values above 10^3. This finding can be understood by the following reasoning: For a system to exhibit catch-bond behavior, it must undergo a transition from sequential to simultaneous pili force exposure (which requires $\eta_x < 1$). The transition takes place when $< L_{II}(F) >$ becomes comparable to Δs_{12}. In addition, the effect this transition has on the lifetime of a system depends on the force at which it takes place; it is more significant at high than at low forces, and in fact does

not take place for the lowest forces (although the force response will deviate from the $\exp(-F/F_{AR})$ dependence indicative of pure sequential pili force exposure). This sets some requirements on η_k for a given η_x. A high value of η_x (although below unity, i.e., for $x_{AR}^{AT} \lesssim x_{LL}^{AT}$) implies that $< L_{II}(F) >$ increases slowly with force, which in turn implies that a significant force is needed to extend $< L_{II}(F) >$ substantially. The transition will in this case take place at sufficiently high forces even for low η_k values. In contrast, if η_x is small (i.e., for which $x_{AR}^{AT} \ll x_{LL}^{AT}$), $< L_{II}(F) >$ increases rapidly with force. For the transition to take place at sufficiently high forces for catch-bond behavior to take place, k_{LL}^{th} needs to be small, which in turn implies that η_k needs to be large. In addition, as was also alluded to in connection to Fig. 5, the relative catch-bond effect is remarkably similar for the two cases (although it is decreasing with decreasing $\overline{\Delta}s_{12}$, approaching zero as $\overline{\Delta}s_{12} \to 0$).

Single-pilus investigations by force-measuring optical tweezers have revealed that the PapG-galabiose AR bond of P pili expressed by *E. coli* is a slip bond (32). On the other hand, based on rolling measurements performed in flow chambers, it has been suggested that the bacterial adhesion might possess catch-bond behavior (12). It was found that rolling adhesion could transit into firm stationary adhesion as the shear stress increases. Since $\eta_x = 0.66$ (thus < 1) for P pili (30), and such experiments allow for multipili attachment, it is in principle possible that there exists a catch-bond behavior in P pili bacteria exposed to rolling that originates from the pili-cooperativity phenomenon studied in this work. However, it has been found that P pili have an η_k value of 0.0033 (23,24,32), which is close to the estimated lower limit for catch-bond behavior in dual-pili-adhering bacterial systems (i.e., $\sim 10^{-3}$). This suggests that dual-pili-adhering P pili-mediated bacteria are unlikely to exhibit catch-bond behavior. On the other hand, since studies based on multipili adhesion have shown that catch-bond behavior can take place under conditions different from those presented in this work, we cannot rule out the possibility that catch-bond behavior can take place for multipili-adhering P pili expressing bacteria. The reason for the transformation of rolling adhesion to firm stationary adhesion of *E. coli* expressing P pili, as reported by Nilsson et al. (12), remains to be determined by further analysis.

In contrast, stronger evidence has been presented (again based on flow chamber measurements) that the type 1 pilus AR bond, FimH-monomannose, is a catch bond (11,12,20,21). This work suggests that the catch-bond behavior observed in type 1-mediated, multipili-adhering bacterial systems might originate from pili cooperativity. Since the bacterial catch-bond effect is enhanced when the thermal opening rate for the LL bonds, k_{LL}^{th}, is low (which implies that η_k is large), and it has been found that k_{LL}^{th} takes a significantly lower value for type 1 than for P pili ($0.016\ s^{-1}$ vs. $0.8\ s^{-1}$ respectively (26)), it is plausible that the intrinsic properties of this bond are such that type 1 pili can exhibit a catch-bond behavior in multipili-adhering bacteria even if the FimH-monomannose is of the slip-bond type. This implies that the observation of catch-bond behavior of rolling type 1 expressing bacteria is not irrefutable proof that the FimH-monomannose bond is a catch bond. The work presented here cannot explain any possible catch-bond behavior from individual type 1 pili; however, preliminary indications of such behavior on a single-molecule level were recently reported (33). It is plausible that the AR bond is a catch bond while at the same time bacterial catch-bond behavior arises from cooperativity. This is an interesting concept that requires further investigation.

CONCLUSIONS

Helix-like pili have unique force-versus-elongation properties that allow for strong pili cooperativity. It has been shown that the adhesion lifetime of multipili-adhering bacteria expressing such pili depends strongly on the internal biomechanical properties of the individual pili (30). Here we have shown, by an analytical treatment as well as by simulations of dual-pili-adhering systems, that the ability of pili to elongate under exposure to stress, and in particular the cooperativity of pili, can provide dual-pili-adhering bacterial systems with a catch-bond behavior even if all bonds are of the slip-bond type. Such a bacterial system has two limiting modes of adhesion: 1), sequential pili force exposure (when one pilus is exposed to the entire force until it detaches, and the remaining pilus then becomes exposed to the entire force, for which there is no cooperativity, which gives rise to a short bacterial adhesion lifetime); and 2), simultaneous pili force exposure (when the force is shared equally between the pili with high cooperativity, which in turn gives rise to a long bacterial adhesion lifetime). Bacterial catch-bond behavior may occur because a bacterium can transit from sequential (mode 1) to simultaneous (mode 2) pili force exposure as the force increases. This can take place if the expected uncoiling length increases with force, which it does for systems in which the bond length of the AR length is shorter than that of the LL bond, i.e., $x_{AR}^{AT} < x_{LL}^{AT}$ or $\eta_x < 1$.

Catch-bond behavior is not a phenomenon that is confined solely to a restricted set of parameter values; rather, it can take place for a large range of values of η_x(still under the condition that $\eta_x < 1$). For each η_x value, there is a certain limit for η_k above which the catch-bond behavior can appear. On the other hand, whenever it takes place, it does so solely within a certain force interval. However, the prolongation of the bacterial adhesion lifetime and the force interval in which the bacterial catch-bond behavior takes place depend on η_x, since it determines the likelihood of mediating the adhesion by simultaneous pili force

exposure during an elongation. For low values of η_x, the catch-bond behavior takes place for significantly lower forces and longer lifetimes than for high η_x values.

It is possible that the lifetimes for which this effect has been predicted to take place in this work do not agree quantitatively with all observed phenomena. Any such discrepancy can be primarily attributed to the fact that this work solely considers dual-pili-adhering systems, whereas bacteria in vivo presumably can adhere to their host by a multitude of pili. Further analyses (presently ongoing) based on multipili adhesion show that catch-bond behavior can take place under conditions other than those presented here, primarily giving rise to longer lifetimes. However, since a multipili study is rather extensive, the results will be reported elsewhere. The qualitative results from this dual-pili-adhering system are also assumed to be valid in the general case of multipili bacterial adhesion.

Finally, we want to stress that this work is not intended to disprove or replace existing theories or explanations regarding catch-bond behavior in various systems; rather, we present a complementary model that shows that bacterial catch-bond behavior can occur in multibinding systems adhering by helix-like pili binding by slip bonds. It is plausible that similar phenomena can also take place in other types of systems. What is needed is a transition from a sequential to a simultaneous binding system, or from a configuration expressing low cooperativity to one that displays a high cooperativity. Understanding this catch-bond phenomenon will help elucidate the dependence of shear stress on the adhesion of rolling bacteria.

SUPPORTING MATERIAL

One table is available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)00709-5.

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