For catch bonds, it all hinges on the interdomain region

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Tensile mechanical force was long assumed to increase the detachment rates of biological adhesive bonds (Bell, 1978). However, in the last few years, several receptor–ligand pairs were shown to form "catch bonds," whose lifetimes are enhanced by moderate amounts of force. These include the bacterial adhesive protein FimH binding to its ligand mannose (Thomas et al., 2002; Thomas et al., 2006), blood cell adhesion proteins P- and L-selectin binding to sialyl Lewis X (sLe^X)–containing ligands (Marshall et al., 2003; Evans et al., 2004; Sarangapani et al., 2004), and the myosin–actin motor protein interaction (Guo and Guilford, 2006). The structural mechanism behind this counterintuitive force–enhanced catch bond behavior is of great interest.

Two newly published papers by independent groups (see Lou et al., 2006 on p. 1107 of this issue; Phan et al., 2006) enhance our structural understanding of how selectins might form catch bonds. Both studies were motivated by differences in the unliganded versus the liganded crystal structures of the N-terminal carbohydrate ligand–binding, or lectin, domain and a neighboring EGF-like domain of P-selectin (Fig. 1). The unliganded P-selectin structure was crystallized with the hinge between the two domains in a "closed angle" or "bent" conformation, whereas the ligand-bound structure showed the hinge in an "open angle" or "extended" conformation (Somers et al., 2000). The authors hypothesized that P-selectin had two states, low and high affinity. It had previously been hypothesized that force would favor the extended putative high-affinity conformation (Konstantopoulos et al., 2003), which could provide a structural mechanism for selectin catch bonds. However, there was no evidence before these recent publications (a) that the extended conformation is longer lived or of higher affinity, (b) that altering the regulation of the hinge region affects catch bond behavior, or (c) for a specific explanation of how extension in a regulatory region would affect binding of ligand by the lectin domain. Thus, the notion of interdomain regulation for selectins remained hypothetical.

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Abbreviations used in this paper: PSGL, P-selectin glycoprotein ligand; sLe^X, sialyl Lewis X; SPR, surface plasmon resonance.

The first of the two recent papers (Phan et al., 2006) showed that the extended state of P-selectin does have a higher affinity for ligand, as previously hypothesized. The authors added a glycosylation site in the hinge region that was predicted to wedge the interdomain hinge open to stabilize the extended conformation. This wedge mutant caused a fivefold increase in the affinity of soluble P-selectin for immobilized P-selectin glycoprotein ligand (PSGL)-1 in surface plasmon resonance (SPR) experiments. It also enhanced adhesion between P-selectin–expressing cells and cells expressing the sLe^X -containing selectin ligand PSGL-1 in both static and flow assays. Interestingly, in both SPR and flow experiments, the mutation decreased the rate of bond formation, but decreased bond detachment rates even more. The authors also predicted that adhesion via L-selectin would be enhanced by a mutation (N138G) in the interdomain region that eliminates a hydrogen bond that favors the bent (putative low-affinity) conformation. This hypothesis was validated with experiments that showed a reduced velocity of L-selectinN138G–expressing cells rolling over PSGL-1–coated surfaces, but measurements of bond on- or off-rates were not reported. Although there is no direct evidence that either mutation does, indeed, favor the extended conformation, the two mutations collectively offer convincing evidence that the extended state has higher ligand affinity. The authors hypothesized that this provides a mechanism to explain catch bonds because force would favor the extended conformation of selectin. However, the effect of the mutations on catch bond behavior was not directly tested.

A paper by Lou et al. (2006) determined how the catch bond behavior of L-selectin was affected by the same N138G mutation. When microspheres coated with either the mutant or native L-selectin were washed over ligand-coated surfaces, tether rates increased approximately twofold for the mutant. In addition, this study used single-molecule experiments using a biomembrane force probe to demonstrate that the N138G mutation, indeed, changed the effect of force on bond lifetimes. Although the mutant still formed catch bonds with longer lifetimes at higher force, it did not require as much force to be fully activated. That is, the bonds formed by the N138G mutant had longer lifetimes than the native structures in the low-force "catch" regime, where increased force stabilizes the bond. However, application of sufficient force can weaken even catch bonds, a process called "slipping." Mutant and native structures showed essentially the same behavior in the higher force "slip" regime, where increased force weakens the bonds. This indicates

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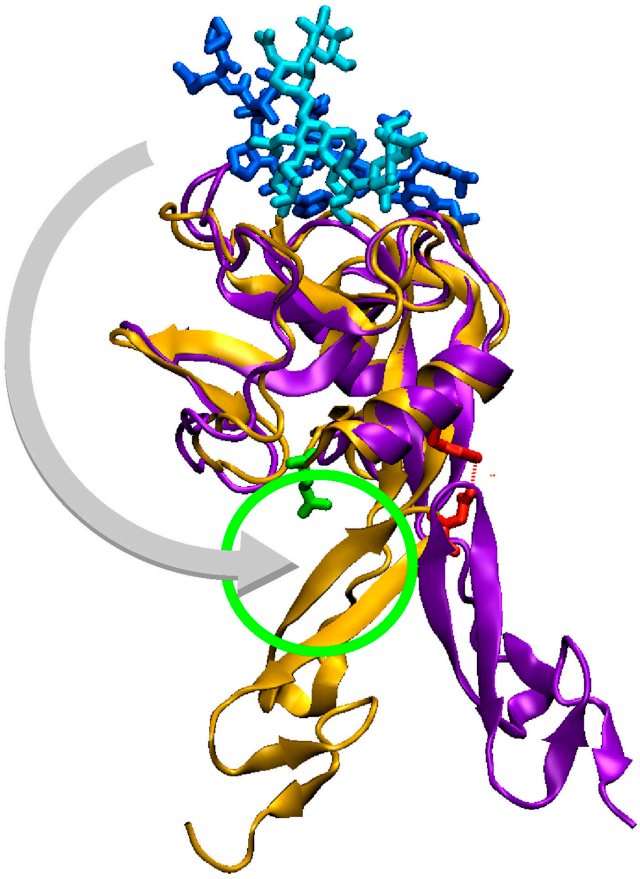

Figure 1. **Conformational changes in P-selectin.** P-selectin was cocrystallized with (purple) and without (gold) the PSGL-1 ligand (peptide, blue; sLe^X, cyan). The two structures differ in a series of changes that span from the binding site to the hinge region, indicated by the arrow. Residue 30 is shown in green, with the position of the wedge mutant glycosylation shown as a green circle. Shown in red is residue N138 forming a hydrogen bond with Y37 as they appear in the unliganded L-selectin structure, which is nearly identical in conformation to unliganded P-selectin.

that the mutation affects the process by which force activates L-selectin, demonstrating that the interdomain hinge region is involved in the catch bond mechanism.

The hypothesis, proposed by both studies, that catch bonds arise as a result of extension of the interdomain region, is in agreement with a previously advanced hypothesis for the mechanism of FimH force-enhanced binding to mannose for bacterial adhesion (Thomas et al., 2002). That work used steered molecular dynamics simulations and site-directed mutagenesis to predict that mechanical force would enhance FimH binding to a mannosylated surface by extending a linker chain connecting the terminal lectin and anchoring pilin domains of the adhesin. This would result in an extended interdomain configuration.

Although it is now evident that interdomain regulation is important for catch bonds, it remains unknown how this region, which is located far from the binding pocket in both selectins and FimH, regulates ligand binding. Beyond providing new experimental insights, Lou et al. (2006) propose a novel, quantitative "sliding–rebinding" mechanism for how interdomain opening increases ligand binding under force. In their model, the ligand is forced to slide past an alternate binding site on the reoriented selectin face when selectin adopts the open angle conformation. The transient binding to this site allows enough time for the ligand to rebind in its original site before it diffuses away. Without the force-induced reorientation, the ligand escapes the pocket without pausing at this intermediate binding site, so it has a lower chance of rebinding. The authors hypothesize that this is why force and the N138G mutation both increase the effective ligand binding lifetime in the catch regime.

Can this theory also explain how the wedge mutation in the hinge region would cause a decrease in the detachment rate of soluble selectin in the SPR experiments reported by Phan et al. (2006)? The drag force acting on an isolated nanoscale ligand–receptor complex in SPR experiments is considered nonexistent. In this case, Brownian motion of the ligand and receptor in the local energy landscape will determine the probability that the ligand interacts with the second binding site after detaching from the first. Without external force to bias the direction of Brownian motion, the orientation of the binding interface will not matter. Thus, the sliding–rebinding model does not explain why mutations regulating the hinge region affect the bond off-rates in the absence of force. Unfortunately, the static kinetic assays of Phan et al. (2006) were performed for a different mutation in a different selectin than the catch bond–demonstrating assays of Lou et al. (2006). Further experiments that connect measurements of lifetimes with and without force, as well as further development of the novel sliding–rebinding model to address very low-force regimes, will be critical to understanding whether catch bonds involve affinity regulation.

An alternative allosteric mechanism was developed to explain FimH catch bonds (Thomas et al., 2006). Whereas the sliding–rebinding model assumes that the orientation, but not the structure, of the binding pocket is changed by force, the allosteric model assumes that extension of the interdomain region causes a conformational change in the binding pocket that affects unbinding rates. It is unknown whether this might also be able to explain the selectin data.

In spite of the unanswered questions, these two papers have established that the hinge region regulates the affinity of P-selectin for its ligand (Phan et al., 2006), as well as the catch bond behavior of L-selectin (Lou et al., 2006). These are important steps toward deciphering the structural origin of the counterintuitive phenomenon called catch bonds.

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