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Reversible unfolding of fibronectin type III and immunoglobulin domains provides the structural basis for stretch and elasticity of titin and fibronectin

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ABSTRACT **The elastic protein titin comprises a tandem array of fibronectin type III and immunoglobulin domains, which are structurally similar 7-strand β-sandwiches. A proposed mechanism for stretching titin, by sequential denaturation of individual fibronectin type III–immunoglobulin domains in response to applied tension, is analyzed here quantitatively. The folded domain is ≈4 nm long, and the unraveled polypeptide can extend to 29 nm, providing a 7-fold stretch over the relaxed length. Elastic recoil is achieved by refolding of the denatured domains when the force is released. The critical force required to denature a domain is calculated to be 3.5–5 pN, based on a net free energy for denaturation of 7–14 kcal/mol, plus 5 kcal/mol to extend the polypeptide (1 cal = 4.184 J). This force is comparable to the 2- to 7-pN force generated by single myosin or kinesin molecules. The force needed to pull apart a noncovalent protein–protein interface is estimated here to be 10–30 pN, implying that titin will stretch internally before the molecule is pulled from its attachment at the Z band. Many extracellular matrix and cell adhesion molecules, such as fibronectin, contain tandem arrays of fibronectin type III domains. Both single molecules and matrix fibers should have elastic properties similar to titin.**

The 3000-kDa titin and the related muscle proteins twitchin and projection are composed of tandem repeats of fibronectin type III (FN3) domains, each containing 98–102 amino acids, interspersed with 91–95 amino acid repeats homologous to immunoglobulin (Ig) constant domains (1, 2). The complete sequence of twitchin is known (3), and partial sequence data are available for titin (4). A number of extracellular matrix proteins, such as fibronectin, tenascin, and several collagens, and cell adhesion proteins of the immunoglobulin superfamily contain tandem FN3 repeats (5). The titin molecule is thought to function as an elastic element, keeping the myosin filament centered in the sarcomere (6, 7). If the elasticity of titin is based on the structure of the FN3 domain, all of these proteins should have similar elastic properties.

Soteriou *et al.* (8) recently proposed that the elastic stretching of titin must involve reversible unfolding of individual FN3 and Ig domains. No other mechanism seemed possible to achieve the 4-fold extension of the I-band segment observed in stretched muscle fibers. They presented experimental evidence that titin underwent an abrupt denaturation in guanidine hydrochloride corresponding to a free energy of 10 kcal/mol per domain (1 cal = 4.184 J) and compared this energy to that estimated from passive elasticity of muscle. The present analysis, initiated independently, addresses the same basic mechanism and extends the analysis. Additional approaches are developed here to estimate the distance that each domain can be stretched, to calculate the force required to unravel FN3 domains, and to compare this force to forces developed by motor molecules and sustained by protein–protein bonds.

The atomic structures of FN3 and Ig domains are very similar (9, 10). Each consists of seven β-strands arranged in two sheets. The β-strands of FN3 and Ig can be closely superimposed in a three-dimensional model, with the exception of the C′ strand, which is on different sheets in the two structures. For the present analysis, this difference is not important, and the FN3 structure will be used for diagrams. Extracellular Ig domains usually contain a disulfide bond linking strands B and F. This bond will almost completely eliminate the extensibility of these domains. The FN3 domains of fibronectin and tenascin contain no disulfides nor presumably do the cytoplasmic FN3 and Ig domains of titin (cysteine residues in cytoplasmic proteins are generally reduced), so full extension of these domains should be possible.

The way FN3 domains fit together in tandem repeats was initially predicted from the x-ray crystal structure of a single FN3 domain, combined with electron microscopy of multidomain segments (9) (Fig. 1). This model has recently been confirmed and demonstrated in detail by x-ray crystallography of a two-domain segment from neuroglian (12). Alternate domains are rotated ≈180° and their long axes are tilted 30° relative to the axis of the molecule. The approximately twofold screw axis molecule generates a straight rather than curved rod, and the interface between adjacent domains provides a degree of rigidity. The spacing between domains was estimated from electron micrographs to be 3.2 nm for tenascin and measured to be 3.77 nm for neuroglian. The spacing of domains and the angles of tilt and rotation will differ somewhat for different proteins, but the basic architecture is likely to be similar. For the present discussion, I will use 3.5 nm as the expected spacing of FN3 and Ig domains.

Titin is the largest polypeptide known, with a mass estimated to be 3000 kDa. The limited sequence data show FN3 domains interspersed with Ig domains (2, 4). Electron microscopy shows a long thin filament ≈950 nm long (13). If the titin molecule consisted entirely of 10-kDa FN3–Ig domains, 300 domains would be required to give the 3000-kDa mass. If domains are spaced 3.5 nm, the total length would be 1050 nm. The agreement of this estimate with the actual length seen in electron microscopy argues strongly that titin does consist of a tandem array of ≈300 FN3–Ig domains.

The globular head of titin, at the C terminus of the molecule, is located at the M line, in the center of the myosin filament, and the molecule runs along the myosin filament for 800 nm (4, 7, 14, 15). This myosin-associated segment of titin is presumably not stretched (it seems unlikely that forces could be applied before the myosin is assembled), so 800 nm of the titin molecule will be in the A

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Abbreviations: FN3, fibronectin type III; Ig, immunoglobulin-like.

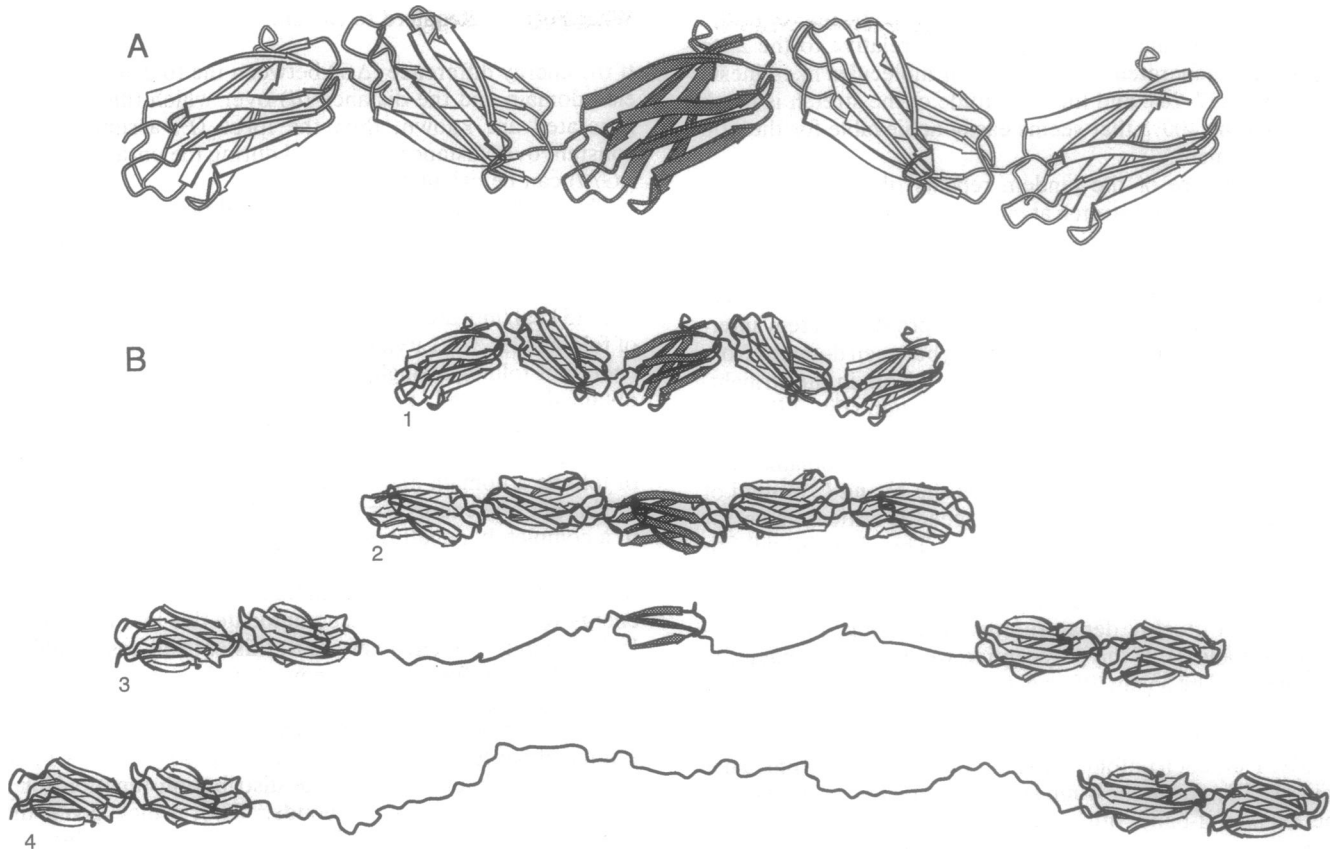

FIG. 1. Structure of tandem FN3 domains and the molecular mechanism of stretching. Five domains are shown in *A* in the zig-zag conformation. The arrowed ribbons indicate the seven β-strands of each domain. In *B* the two stages of stretching are illustrated. Conformation 1 shows the relaxed molecule in the zig-zag conformation; conformation 2 shows the zig-zag straightened out at low force; conformations 3 and 4 show an entire domain unraveling and the polypeptide stretched out, respectively. [This figure was produced by Daniel J. Leahy (Johns Hopkins University School of Medicine) using the program MOLSCRIPT (11) and generously provided for this paper.]

band. This leaves 150 nm of the titin molecule to extend across the half I band to anchor in the Z line (Fig. 2). This is less than the 250- to 400-nm distance for naturally occurring rest-length half I bands and suggests that the I-band titin segment may be already stretched in the rest-length sarcomere. However, the estimated 150-nm length of the I-band titin segment is based on subtraction of two large numbers and may underestimate the actual length. Thus it is possible that titin may span the I band without stretching. If the relaxed titin I-band segment is really 150 nm, it would contain ≈43 domains, some 3–10 of them being stretched. If titin spans a 250- to 400-nm half I-band segment without stretching, this segment would have to contain 70–115 domains.

Elasticity and Stretch: The Structural Basis

The globular domains of protein molecules are generally rigid structures. The Young's modulus of actin filaments and microtubules was recently estimated to be "~1.2 GPa, similar to Plexiglas and rigid plastics" (16). The constituent protein subunits must have a rigidity at least that of the polymer. If the titin filament were a Hookian solid, with a diameter of 2.25 nm, this Young's modulus would require a

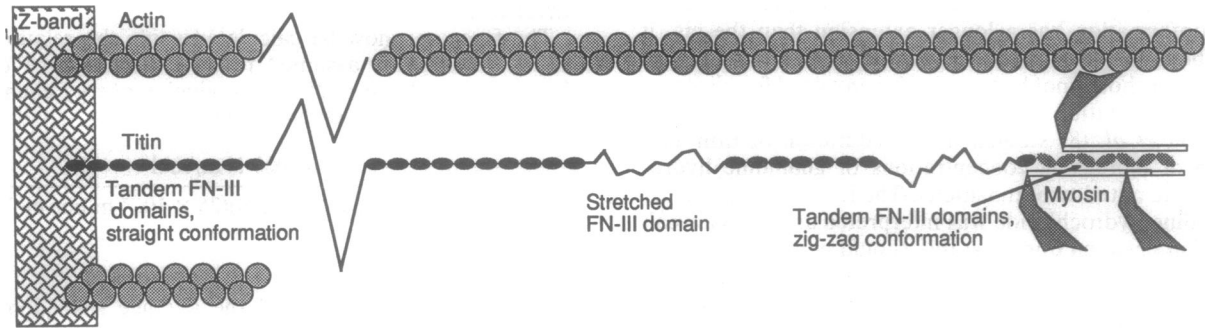

FIG. 2. Diagram showing a single titin molecule extending across the I band, from its attachment at the Z line on the left to its initial association with the myosin rod on the right. The titin molecule continues along the 800-nm myosin rod in the zig-zag conformation. The remaining segment of titin must span the 250- to 400-nm half I band. If this segment of titin is 150 nm, as indicated by electron microscopy length measurement, it would contain about 43 FN3–C2 domains, and 3–10 of these would have to be stretched to span the distance. Alternatively, if the titin I-band segment contained 70–115 domains, it could span the I band without stretch.

force of 50 pN to produce a 1% stretch and 500 pN for a 10% stretch. These numbers are quite large relative to the 2- to 7-pN force generated by single motor molecules like kinesin or myosin (17–20), and the magnitude of the stretch is much less than the 400% that seems easily achievable for the titin I-band segment.

The structure of the tandem repeats of titin shows two features that should stretch at much lower forces (Fig. 1). The first stretch would involve straightening out the zig-zag tilt between domains. This would require disrupting the interfaces between adjacent domains, which are relatively small areas of contact, equivalent to weak protein–protein interactions. This could extend the spacing between domains from 3.5 nm in the zig-zag conformation to 4.0 nm in the straightened conformation, giving a 15% elongation (Fig. 1*B*).

The second and more important mechanism for stretching would involve completely unraveling FN3–Ig domains. The tensile force on titin will operate on the N and C termini of all domains and will eventually exceed a critical value for denaturation of the weakest domain. This domain will initially unravel by peeling off the weakest β-strands, but the remaining structure should be greatly destabilized and the domain will quickly denature completely (Fig. 1*B*, conformations 3 and 4). If the polypeptide were fully extended the length would be 36 nm (95 aa at 0.38 nm per aa). As discussed below, the polypeptide extension at moderate forces will be limited to some fraction of this maximum, estimated to be ≈80%. Thus each unfolded domain will extend to 29 nm, a net gain of 25 nm from the 4-nm spacing of the folded domain. If the relaxed I-band titin segment is 150 nm long, the 43 domains should be extendible to a total length of 1250 nm. If this segment has 70–115 domains (i.e., it is long enough to span the rest-length I band without stretch), extension to 2–3 μm should be possible.

Contraction of the filament when the force is released will occur as the domains spontaneously refold. FN3 domains refold easily when a denaturing solvent is removed (21), and it is reasonable to assume that they would also refold when a denaturing force is removed. An important unresolved question is the kinetics of this refolding. Small proteins and domains refold *in vitro* with complicated multistep kinetics. For example interleukin 1β, which is an all β-sheet protein like the FN3 domain, regains 90% of its secondary structure (as indicated by circular dichroism) in a few milliseconds, but transitions to the full native conformation (as indicated by tryptophan fluorescence and amide hydrogen exchange) require seconds to minutes (22). Refolding of a stretched FN3 domain might proceed rapidly to a molten globule of approximately the 4-nm size, followed by slower consolidation of the complete native structure. The several proline residues in the FN3 domain are all in the trans conformation. Since the trans conformation has a longer extension than the cis, it should be favored in a stretched polypeptide. Therefore, the renaturation should not be retarded by any need for cis–trans proline isomerization.

Soteriou *et al.* (8) studied the denaturation of titin as a function of increasing concentrations of guanidine hydrochloride and noted two transitions. The first transition at 0.1 M guanidine hydrochloride was interpreted as due to a weak interaction between domains; this should correspond to the transition from zig-zag to straight conformation in Fig. 1. The second transition was a rather abrupt and complete loss of β-sheet secondary structure centered at 1.3 M guanidine hydrochloride, which was interpreted as the approximately simultaneous unfolding of all domains. All domains may be similarly stable, producing a single unfolding step rather than a broad distribution. The free energy for unfolding a single domain was estimated to be 10 kcal/mol.

What Force Is Required to Denature and Stretch a Domain?

If the energy difference (ΔG) between the folded and unraveled domain and the distance (d) over which this energy is generated are known, then the force (F; assumed to be constant over distance d) exerted during the unraveling ($F = \Delta G/d$) can be calculated.

The net free energy for folding small proteins or domains has been determined for several proteins by measuring the fraction of folded protein in denaturing urea or guanidine solutions and extrapolating to zero urea. Values in the range of 5–15 kcal/mol are typically obtained for the net free energy of folding (23, 24). Litvinovich *et al.* (21) determined the free energy of folding a single FN3 domain from fibronectin and obtained a value of 7 kcal/mol. Soteriou *et al.* (8) determined that all domains of titin unfold at about the same free energy, 10 kcal/mol. I will use 10 kcal/mol as the average free energy of folding a domain and consider a range of 7–14 kcal/mol.

This net free energy for folding, 10 kcal/mol, is referenced to a completely denatured polypeptide. If the polypeptide is stretched, however, it will have fewer available conformations than the completely denatured chain. Additional free energy will be required to accommodate this loss in entropy. If the chain is stretched very taut, this entropy loss could be substantial, but for a less extreme stretch, it is probably less important. A quantitative analysis of this is beyond the scope of the present work, but the magnitude of the effect can be estimated based on the analysis of Flory (25). Figures 1 and 6 in Flory's chapter 8 show the distribution function for a freely jointed chain of 10 bonds. $W(r)$, which gives the number of conformations for an end-to-end spacing (r), decreases progressively as r increases. $W(r)$ is in the range of 10^{-3} for 30–50% of maximum extension and falls to 10^{-7} at 80% maximum extension. If we estimate that 1/10,000 of the total conformations correspond to r between 80% and 100% extension, the free energy associated with this entropy loss will be $\Delta G = -T\Delta S = -RT\ln(1/10{,}000) = 5.5$ kcal/mol. This extrapolation is somewhat speculative but makes two important points. (*i*) The entropic effect becomes important as the chain is stretched to 50–80% of its maximum extension, and (*ii*) the effect is only a few kcal/mol for an extension that reduces the number of conformations by several orders of magnitude. To accommodate this entropic effect, I will assume that the polypeptide of the unraveled domain is only stretched to 80% of its maximum extension (i.e., 29 nm instead of the maximum possible 36 nm), and I will add 5 kcal/mol to the free energy required to denature the domain, to account for the energy required to extend the polypeptide. Thus the total free energy required to denature the domain and stretch it to 80% of maximum extension will be 15 kcal/mol. These numbers are likely to be accurate within a factor of 2.

The force can now be calculated using the relation $F = \Delta G/d$, where F is assumed to be constant force over a distance $d = 29 - 4 = 25$ nm, producing energy change $\Delta G = 15$ kcal/mol = 63,000 N·m/mol.

$$F = \frac{63{,}000\ \text{N·m/mol}}{(6 \times 10^{23}\ \text{molecules per mol})(25 \times 10^{-9}\ \text{m per molecule})}$$

$$= 4 \times 10^{-12}\ \text{N}. \qquad [1]$$

The force required to unfold the average FN3 domain is therefore estimated to be 4 pN. If the stability of the domains ranges from 7 to 14 kcal/mol, they will be successively unfolded as the force is increased from 3.5 to 5 pN.

The elasticity of titin should be very different from a Hookian spring. There should be a 15% extension at low force (transition from the zig-zag to straight conformation)

and then no further extension until the yield point of ≈3.5 pN is reached. After this yield point, the entire molecule should unravel and stretch up to 700% with only a small increase in force to ≈5 pN.

Experimental Observations of Titin Stretching and Tension Generated

It seems well established that the I-band segment of titin does stretch during sarcomere elongation. Using an antibody against an epitope in this segment, Horowits *et al.* (7) found that its distance from both the A band and Z line increased in direct proportion as the half I-band length was stretched from 250 to 1000 nm. This means that the stretching was distributed along the filament on both sides of the epitope. Trombitas *et al.* (26) used an antibody against a titin epitope located about one-third the distance from the end of the A band to the Z line. Muscle was frozen in an extended state, fractured somewhere in the I band, thawed to allow elastic filaments to contract, and labeled with antibody. If the fracture was on the Z-band side of the epitope, the label was found to contract all the way to the A band. When the fracture was on the A-band side, the epitope contracted to the Z band, but it did not go all the way. It formed a distinct band ≈100 nm from the Z band. As discussed above, the unstretched titin I-band segment may be only 150 nm long, in which case the antibody label should be 50 nm from the A band or 100 nm from the Z band in the unstretched titin. The 50-nm spacing from the A band was too close to resolve in the micrographs, and the 100-nm spacing from the Z band was exactly what was observed. These two epitope mapping studies are therefore completely consistent with the model of titin elasticity proposed here and with the conclusion that the unstretched titin I-band segment may be only 150 nm.

Wang *et al.* (27) determined stress–strain curves for split fibers of rabbit skeletal muscle in relaxing solution. No significant passive tension was observed until the muscle was stretched significantly (600 nm per half I band for psoas muscle), after which tension rose steeply to a plateau at 1000 nm per half I band. However, in a subsequent study, Granzier and Wang (28) eliminated the actin thin filaments and noted passive tension of psoas muscle at all sarcomere lengths beyond a 150-nm half-I-band length. This is consistent with the 150-nm titin I-band segment, but it could also arise from more complex mechanisms with a longer titin I-band segment.

Wang *et al.* (27) estimated the peak tension to be 150 pN per thick filament for psoas muscle. This gives a peak tension of 25 or 50 pN per titin, depending on whether one assumes six or three titins per half thick filament (8, 27). This tension is much higher than the 5-pN maximum elastic force predicted here. Soteriou *et al.* (8) also concluded that the peak tension was larger than could be accounted for by the denaturation energy. There are several possible explanations for the discrepancy at the maximum extension of the sarcomere, but it is perhaps more relevant to look at the force at low or intermediate stretch. The data of Granzier and Wang (28) are probably the most relevant, but they were not extrapolated to single thick filaments or single titins. Nevertheless, comparing these curves to the previous study suggests that the passive tension observed at modest stretch in the absence of actin is probably much closer to the predicted 3.5–5 pN.

Stretch Response of Fibronectin and Related Proteins

Soteriou *et al.* (8) noted that this mechanism of elasticity may have broader applications to other proteins with FN3 domains, mentioning in particular the Ig family of adhesion molecules such as neural cell adhesion molecule. The five Ig domains of the neural cell adhesion molecule will not be extendible because of the disulfide bond linking the B and F strands, but the two FN3 domains could be stretched 50 nm beyond their rest length. Elasticity may be especially important for fibronectin, a dimeric molecule with a length of 160 nm when fully extended but not stretched (29). The 28 FN3 domains in the two subunits of plasma fibronectin should extend the molecule to a length of $160 + 28 \times 25 = 860$ nm if subjected to a force of ≈5 pN. Moreover, the molecule should renature and recoil when the force is released. Fibronectin matrix fibrils, which probably consist of parallel staggered arrays ≈10 molecules thick (30), should also be very extendible, at proportionally larger forces of ≈50 pN. The elasticity of fibronectin may play an important role in the structure and plasticity of the extracellular matrix.

The Force Generated by a Single Motor Molecule Can Stretch Titin

The magnitude of the force required to stretch titin or fibronectin must be put in context of the forces operating in cells. In recent studies the force generated by a single kinesin on a microtubule was measured to be 2 (17) or 5 (18) pN. The force generated by single myosin molecules on an actin filament averaged 3 pN but was sometimes as high as 7 pN (20). Thus the force generated by a single motor molecule is similar to the threshold for denaturing FN3 domains, and two to four motors operating in parallel should stretch titin or fibronectin completely.

What Force Is Required to Pull Apart a Protein–Protein Interface?

The above calculation of the force to denature a protein domain, based on the formula $F = \Delta G/d$, is similar to the simplest calculation by Bell (31) of the force that would be required to rupture a noncovalent protein–protein interface (I will refer to this interface as the protein–protein bond), in equation 15 in ref. 31. However, for a reversible protein–protein bond, it is more meaningful to calculate the lifetime of the bond and how this is decreased by applied force. Bell's equation 16 is rewritten and modified here:

$$\tau(F) = \tau_o \exp(\Delta G^{bond} - dF)/kT = \tau(0) \exp(-dF/kT). \quad [2]$$

$\tau(F)$ is the lifetime of the bond subjected to force F, and $\tau(0)$ is the lifetime at zero force [$\tau(0) = 1/k_{-1}$, where k_{-1} is the dissociation rate constant for the unstressed bond]. The parameters τ_o and ΔG^{bond} are incorporated in $\tau(0)$ and are not needed here. d is the distance over which the bond energy is dissipated (the hydrophobic forces of the protein–protein bond operate over a distance about the size of a water molecule, so $d = 0.3$ nm is a reasonable value). F is the applied force, assumed to be constant over the distance d, k is Boltzmann's constant, and T is 300 K.

The calculation will be applied to bonds of two strengths and lifetimes. The first will be a typical receptor–ligand bond, with $K_d = 10^{-9}$ M, $k_2 = 2 \times 10^6$ M^{-1}·s^{-1} [the diffusion-limited association rate for protein–protein association (32)] and $k_{-1} = 2 \times 10^{-3}$ s^{-1}, giving $\tau(0) = 1/k_{-1} = 500$ sec. The second is the much stronger bond that holds the actin filament together. The total bond that must be broken to fragment an actin filament is estimated to have the following parameters: $K_d = 10^{-13}$ M, $k_2 = 10^5$ M^{-1}·s^{-1}, and $k_{-1} = 10^{-8}$ s^{-1} (33), giving $\tau(0) = 10^8$ sec. By using Eq. 2, the lifetimes are plotted in Fig. 3 as a function of increasing force.

Regardless of the initial strength of the bond, its lifetime is reduced a factor of 2 by a 10-pN force and a factor of 10 by a 30-pN force. Every 30-pN increment in the applied force reduces the lifetime of the bond another factor of 10. Thus if fibronectin were attached to an integrin receptor and subjected to tension by contractile events in the cell, the fibronectin

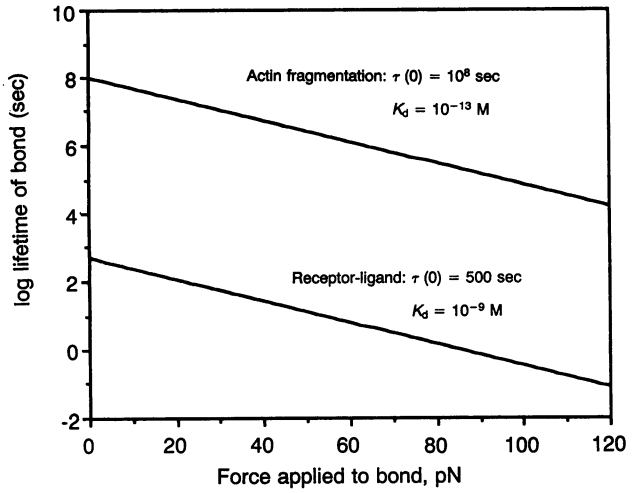

FIG. 3. Lifetime of a noncovalent protein–protein interface subjected to increasing force is plotted on a logarithmic scale. Data for the very high-affinity bond that must be broken to fragment an actin filament, with a lifetime $\tau(0) = 10^8$ sec in the absence of force, and a high-affinity bond typical of receptor–ligand pairs, with $K_d = 10^{-9}$ M and a lifetime of 500 sec, are shown. A force of 10–30 pN reduces the lifetime of each bond by a factor of 2–10.

filament would stretch and unravel at a 3.5- to 5-pN force, while the bond holding the integrin to the fibronectin would not be weakened substantially until the force reached 10–30 pN.

For actin, it is interesting to compare the calculation to the 120-pN force that Kishino and Yanagida (34) measured as the tensile strength of an actin filament. At 120 pN, the lifetime of a bond within the actin filament is calculated here to be 17,000 sec. Since actin subunits are spaced 5.5 nm apart, there will be 1800 bonds in a 10-μm filament, and the lifetime of the filament (the time needed to break any one bond) should be $\approx$10 sec. If the force is reduced to 60 pN, the lifetime of the individual bond is increased to 1,300,000 sec and the lifetime of the filament would be 720 sec. One would therefore expect to see filaments break readily at 120-pN force but break only rarely at 60 pN, in good agreement with the experimental observation.

A similar comparison is now available for the very high-affinity biotin–avidin complex. Florin *et al.* (35) used atomic force microscopy to disrupt biotin–avidin complexes and concluded that a force of 160 pN was needed to disrupt a single biotin–avidin bond. The dissociation rate in the absence of force has been reported to be $k_{-1} = 0.4 \times 10^{-7}$ sec^{-1} (36) giving $\tau(0) = 2.5 \times 10^7$ sec. At 160 pN force, Eq. 2 predicts the lifetime will be reduced to 200 sec. Although the time course of bond disruption was not investigated in the study by atomic force microscopy, observable disruption of the bonds would require that the lifetime be reduced to seconds or minutes. The 200-sec calculated lifetime is within an order of magnitude of what must have been obtained in the experiment.

A noncovalent protein–protein bond is able to support a force substantially larger than that generated by a motor molecule. This is, of course, essential for the operation of motor molecules, since their force production is produced through protein–protein bonds. Less obvious but just as interesting is the conclusion that the domains within a molecule like titin or fibronectin can be stretched and denatured by weaker forces than are required to pull two subunits apart. The most important factor in this difference is the distance over which the force operates. The protein–protein bond operates over 0.3 nm, while the domain stretching extends over 25 nm. I conclude that titin, fibronectin, or other molecules with similar domains will stretch internally long before they are pulled away from the proteins to which they are attached.

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1. Trinick, J. (1991) *Curr. Biol.* **3**, 112–119.
2. Higgins, D. G., Labeit, S., Gautel, M. & Gibson, T. J. (1994) *J. Mol. Evol.* **38**, 395–404.
3. Benian, G. M., L'Hernault, S. W. & Morris, M. E. (1993) *Genetics* **134**, 1097–1104.
4. Labeit, S., Gautel, M., Lakey, A. & Trinick, J. (1992) *EMBO J.* **11**, 1711–1716.
5. Bork, P. & Doolittle, R. F. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 8990–8994.
6. Horowits, R., Kempner, E. S., Bisher, M. E. & Podolsky, R. J. (1986) *Nature (London)* **323**, 160–164.
7. Horowits, R., Maruyama, K. & Podolsky, R. J. (1989) *J. Cell Biol.* **109**, 2169–2176.
8. Soteriou, A., Clarke, A., Martin, S. & Trinick, J. (1993) *Proc. R. Soc. London B* **254**, 83–86.
9. Leahy, D. J., Hendrickson, W. A., Aukhil, I. & Erickson, H. P. (1992) *Science* **258**, 987–991.
10. Main, A. L., Harvey, T. S., Baron, M., Boyd, J. & Campbell, I. D. (1992) *Cell* **71**, 671–678.
11. Kraulis, P. J. (1991) *J. Appl. Crystallogr.* **24**, 946–950.
12. Huber, A. H., Wang, Y. E., Bieber, A. J. & Bjorkman, P. J. (1994) *Neuron* **12**, 717–731.
13. Nave, R., Fürst, D. O. & Weber, K. (1989) *J. Cell Biol.* **109**, 2177–2187.
14. Fürst, D. O., Osborn, M., Nave, R. & Weber, K. (1988) *J. Cell Biol.* **106**, 1563–1572.
15. Itoh, Y., Suzuki, T., Kimura, S., Ohashi, K., Higuchi, H., Sawada, H., Shimizu, T., Shibata, M. & Maruyama, K. (1988) *Biochem. J.* **104**, 504–508.
16. Gittes, F., Mickey, B., Nettleton, J. & Howard, J. (1993) *J. Cell Biol.* **120**, 923–934.
17. Kuo, S. C. & Sheetz, M. P. (1993) *Science* **260**, 232–234.
18. Svoboda, K., Schmidt, C. F., Schnapp, B. J. & Block, S. M. (1993) *Nature (London)* **365**, 721–727.
19. Huxley, A. F. & Simmons, R. M. (1971) *Nature (London)* **233**, 533.
20. Finer, J. T., Simmons, R. M. & Spudich, J. A. (1994) *Nature (London)* **368**, 113–119.
21. Litvinovich, S. V., Novokhatny, V. V., Brew, S. A. & Ingham, K. C. (1992) *Biochim. Biophys. Acta* **1119**, 57–62.
22. Varley, P., Gronenborn, A. M., Christensen, H., Wingfield, P. T., Pain, R. H. & Clore, G. M. (1993) *Science* **260**, 1110–1113.
23. Fersht, A. R. & Serrano, L. (1993) *Curr. Opin. Struct. Biol.* **3**, 75–83.
24. Privalov, P. L. (1979) *Adv. Protein Chem.* **33**, 167–241.
25. Flory, P. J. (1969) *Statistical Mechanics of Chain Molecules* (Interscience, New York).
26. Trombitas, K., Pollack, G. H., Wright, J. & Wang, K. (1993) *Cell Motil. Cytoskeleton* **24**, 274–283.
27. Wang, K., McCarter, R., Wright, J., Beverly, J. & Ramirez-Mitchell, R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7101–7105.
28. Granzier, H. L. M. & Wang, K. (1993) *Biophys. J.* **65**, 2141–2159.
29. Erickson, H. P., Carrell, N. A. & McDonagh, J. (1981) *J. Cell Biol.* **91**, 673–678.
30. Singer, I. I. (1979) *Cell* **16**, 675–685.
31. Bell, G. I. (1978) *Science* **200**, 618–627.
32. Northrup, S. H. & Erickson, H. P. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3338–3342.
33. Erickson, H. P. (1989) *J. Mol. Biol.* **206**, 465–474.
34. Kishino, A. & Yanagida, T. (1988) *Nature (London)* **334**, 74–76.
35. Florin, E.-L., Moy, V. T. & Gaub, H. E. (1994) *Science* **264**, 415–417.
36. Green, M. N. (1990) *Methods Enzymol.* **184**, 51–67.