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Influence of Architecture on the Kinetic Stability of Molecular Assemblies

Amesh B. Patel, Stephanie Allen, Martyn C. Davies, Clive J. Roberts, Saul J. B. Tendler, and Philip M. Williams*

Laboratory of Biophysics and Surface Analysis, School of Pharmacy, The University of Nottingham, Nottingham NG7 2RD, U.K.

Received June 16, 2003; E-mail: phil.williams@nottingham.ac.uk

Maintenance of a system at its energetically ideal state (folded protein or receptor−ligand complex) is dictated by the lifetime, and this is dependent on the magnitude of the energy barrier required to overcome the transition between states. Dynamic force spectroscopy (DFS)[1−3] has been used to yield energy landscape parameters, including barrier locations, as well as times needed to traverse these energy barriers.[3−6] Estimation of the "off-rate" over a particular kinetic barrier requires extrapolation of its behavior under various forces to zero force. For single bonds/interactions (i.e., where only one receptor ligand complex is broken or one protein unfolded in each test of strength), this is a reasonable method of off-rate calculation, especially because great efforts are often made to obtain such single molecule events.[7] Extension of the theory to account for the presence of multiple instances of the molecular "bond" has been shown to have a subtle effect on the failure kinetics of the complete system.[2,7,8] This effect of discrete molecular attachments on the failure kinetics of a multimolecular assembly has yet to be shown experimentally by DFS. The data presented here show the effect of such multiple attachments on the dynamic force spectrum of 2′-iminobiotin (IB), using atomic force microscopy (AFM) as the force probe.

Single specific adhesion events were measured between a ligand (IB) attached to an AFM tip (as the IB−bovine serum albumin (BSA) conjugate) and streptavidin (SA). SA was immobilized on a 3-aminopropyldimethoxysilane-treated silicon substrate via an interaction with either biotin (B)−BSA or IB−BSA immobilized through glutaraldehyde (Figure 1).[9] The loading rate was varied over 2 orders of magnitude between 1000 and 100 000 pN s^{-1} by changing the AFM tip retraction rate from the sample (180−3000 nm s^{-1}), and by using cantilevers with different spring constants (10−90 pN nm^{-1}). Several hundred unbinding events were measured at each rate of force loading, and the most probable rupture force for each rate was determined as the mode of the force distributions. The dynamic force spectra (Figure 2) for IB with SA immobilized by either B−BSA (blue ●) or IB−BSA (green △) each indicate two linear force regimes, with thermal force scales ($f_\beta = k_B T/x_\beta$) of ∼7 and ∼30 pN. These two regimes indicate the presence of two transition states along with unbonding trajectory of the system, located at a projected distance x_β of 0.6 and 0.14 nm.

Measurements of transition state displacements of 0.14 and 0.6 nm are in close agreement with those seen in other experimental[3,10,11] and molecular simulation[12−14] studies of the B−SA interaction. This agreement between the locations of the energy barrier determined experimentally and those predicted through simulation permits the latter to be used to indicate the chemical origins of the forces measured.[12,14−16] The ability to reduce the frequency of adhesion events between the probe and substrate by blocking the system with excess biotin also confirms that the measurements are that of the specific receptor ligand complex and are not due to nonspecific adhesion nor removal of the BSA conjugate from the tip or substrate.

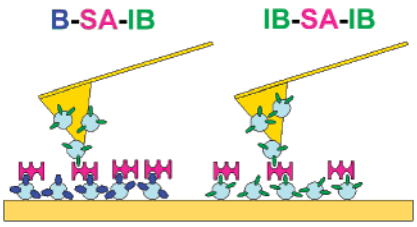

Figure 1. The failure rate of two molecular architectures, biotin−streptavidin−iminobiotin (B−SA−IB) and iminobiotin−streptavidin−iminobiotin (IB−SA−IB), was examined by force spectroscopy.

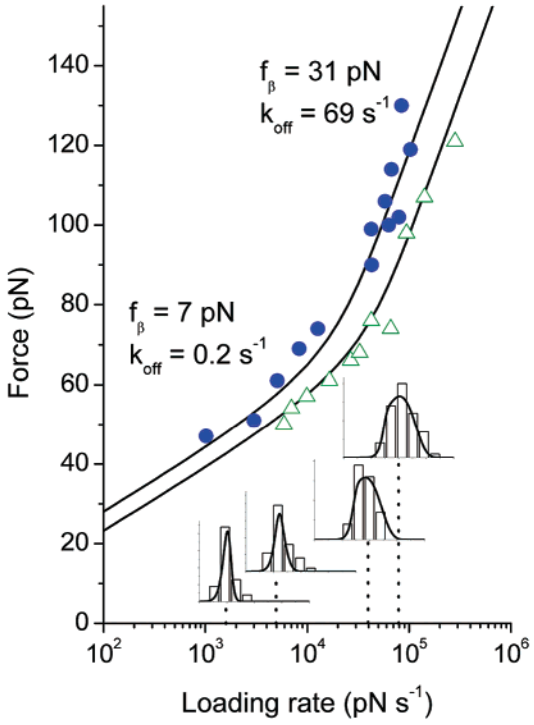

Figure 2. The dynamic force spectrum of IB−SA−IB (green △) is clearly displaced to higher off-rates than that of B−SA−IB (blue ●). The solid lines are fits of the model assuming a doubling of off-rate for two bonds. The off-rates and force scales are given for the B−SA−IB system. The insets are predicted force histograms at various loading rates overlaid on those measured.

The dynamic force spectrum for the IB−SA−IB arrangement is translated to the right of that of B−SA−IB; this difference can only be due to the difference in the molecular arrangement. For both systems prior to rupture, there exist two bonds in series. Here, the loading rate experienced by each bond is the same as that applied to the system. Therefore, most often, the failure of the B−SA−IB

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system will be at the molecular bond with the shortest lifetime, which here is known to be that between IB and SA. The dynamic force spectrum for B−SA−IB is characteristic of a system with one point of failure, the IB−SA bond. Because of the dynamic strength of interactions, there is the possibility that the stronger B−SA bond would sometimes fail. However, this would affect the force distributions only where the dynamic force spectra of the two systems overlap. The effective loading rate on each of the IB−SA bonds of the IB−SA−IB system is the same as the applied loading rate, but now no bond is more likely to break over the other because they both possess the same dynamic strength. There is the possibility that the SA is immobilized to the surface by two IB−SA bonds; the combination of size of the BSA protein and the average substitution level of approximately 11 IB-per-protein suggest that the propensity of doubly attached SA proteins will be low and hence not affect the mode of the force distribution. Hence, with two identical points of breakage, the chance of failure per unit time of any IB in IB−SA−IB should be greater than that of the single IB in B−SA−IB by a factor of ∼2.

A model of unbonding kinetics across two transition states was fitted to the dynamic force spectra by simplex minimization.[3,17] This analysis of the B−SA−IB data reveals kinetic off-rates of 0.2 and 54 s^{-1} across the two barriers measured. Similarly, the IB−SA−IB data suggest kinetic off-rates of 0.1 and 128 s^{-1}. Experimentally, more data were obtained for the high force-scale regime of B−SA−IB and the low force-scale regime of IB−SA−IB; this accounts for the difference in spread between spectra. Fitting of both spectra together, by assuming the force scales of both architectures are equal, indicates that the failure rate for one and two IB−SA bonds in series varies by a factor of 2.6. Forcing the force scales of IB−SA−IB and B−SA−IB to be equal, and also the ratio of both off-rates to be 2, gives force scales of 6.8 and 30.7 pN and rates (for IB−SA) of 0.2 and 69 s^{-1}.

In agreement with theory,[18] we have demonstrated that the failure rate of a molecular assembly increases with the number of points of failure. This result raises interesting points for consideration. Most often, a molecular assembly will fail under force at the weakest point (the concept of weak versus strong, however, is not simple to define as dissimilar bonds may exhibit different dynamic force spectra).[8] It should also be recalled that dynamic strength under forces above a few piconewtons is not related to the affinity of the interaction because it is dependent solely on the rate of dissociation.[1] The B−SA system is often used as a convenient way to immobilize biomolecules for investigation under force. Assuming the dynamic force spectrum for the molecule under investigation lies below that of B−SA, the tests of strength are satisfactory. However, in the measurements of the B−SA interaction itself, the B−SA bond is used to immobilize the SA protein. Here, there are often two identical bonds loaded in series, a B−SA−B arrangement. Our result above indicates that the failure rate of the B−SA−B system will be twice that of the B−SA bond and hence the kinetic off-rates of this interaction measured by DFS are overestimated by a factor of 2.

We have shown that the kinetic stability of a multimolecular complex arranged in series decreases as the number of points of failure increases. This decrease in a system's lifetime by introducing multiple copies of the bond under study is an interesting method of increasing the efficiency of computational studies. Atomistic molecular dynamics simulations suffer due to the severe restriction on the time available,[13] which is currently submicrosecond using high-performance parallel compute architectures. As an alternative to distributing the compute task, one can simulate concurrently many copies of the bond under study. With two copies, for example, a bond failure event will be seen (on average) in half of the time, representing 100% speedup efficiency. Providing the simulation of each copy of the molecular complex is longer than the time between attempts to unbond (which is related to the diffusional relaxation time of the complex), then such a computing methodology is the most efficient utilization of the resource available. While it is currently possible, therefore, to use this methodology to simulate the kinetics of small ligand unbinding, with relaxation times of a few nanoseconds,[3] accurate simulations of protein folding, with much slower diffusion times,[19,20] are still challenging.[21,22]

We have shown that DFS requires an appreciation of molecular architecture of the system being probed as the kinetic stability depends on the complexity of the molecular architecture. Adjustment of DFS data to account for the effect of multiple connected bonds is needed before extracting meaningful off-rates, and this is applicable to receptor−ligand systems, and to the unfolding of proteins consisting of tandem repeats, such as titin.[17,23]

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