Molecular Mechanisms Determining the Strength of Receptor-Mediated Intermembrane Adhesion

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ABSTRACT The strength of receptor-mediated cell adhesion is directly controlled by the mechanism of cohesive failure between the cell surface and underlying substrate. Unbinding can occur either at the locus of the specific bond or within the bilayer, which results in tearing the hydrophobic anchors from the membrane interior. In this work, the surface force apparatus has been used to investigate the relationship between the receptor-ligand bond affinities and the dominant mechanism of receptor-coupled membrane detachment. The receptors and ligands used in this study were membrane-bound streptavidin and biotin analogs, respectively, with solution affinities ranging over 10 orders of magnitude. With the optical technique of the surface force apparatus, the occurrence of membrane rupture was directly visualized in situ. The latter observations together with measurements of the corresponding intermembrane adhesive strengths were used to identify the dominant failure pathway for each streptavidin-analog pair. Even in cases where the membrane pull-out energy exceeded the equilibrium bond energy, cohesive failure occurred within the membrane interior at nearly all bond affinities considered. These results are consistent with previous findings and provide direct support for the commonly held view that, under nonequilibrium conditions of applied external stress, the gradient of the bond energy, not the equilibrium bond energy alone, determines the adhesive strength. Furthermore, our findings directly demonstrate that, in the presence of competing failure mechanisms, the preferred detachment mechanism—hence, the adhesive strength—will be determined by the bond that exhibits the weakest tensile strength. Because the tensile strength is determined by the gradient of the unbinding energy, the critical detachment force will be determined by both the bond energy and the effective bond length.

INTRODUCTION

The strengths of receptor-mediated adhesive junctions in both biomaterials engineering and in cell adhesion often have been correlated with the energies of the specific receptor-ligand bonds (Evans, 1985; Evans et al., 1988; Kuo and Lauffenburger, 1993; Dembo et al., 1988; Kaplanski et al., 1993). This can only be the case, however, in the absence of competing detachment mechanisms (Evans, 1994). Cell motility and cell adhesion studies, for example, have attempted to determine the strengths of the individual receptor-ligand bonds that support traction forces or resist external shear, and to correlate their tensile strengths with the equilibrium bond energies (Evans, 1985; Evans et al., 1988; Kuo and Lauffenburger, 1993; Dembo et al., 1988; Kaplanski et al., 1993). However, in the latter cell detachment studies, it was often observed that both receptor-ligand bond rupture and disruption of the bilayer membranes contributed simultaneously to cell unbinding (Evans, 1985; Evans et al., 1988; Dembo et al., 1988; Kuo and Lauffenburger, 1993). Clearly, competing detachment mechanisms occur at sites other than the bond loci, and the cell adhesion strength ultimately will be determined by the tensile strengths of both the specific bonds and the additional competing junctions.

Although the tensile strengths of the discreet bonds between covalently immobilized ligands and receptors reportedly demonstrate a logarithmic dependence on their intrinsic affinities (Dembo et al., 1988; Kaplanski et al., 1993; Kuo and Lauffenburger, 1993), when the hydrophobic membrane anchors preferentially tear from the bilayer, the adhesion strength is controlled by the strength of cohesion of the lipid matrix and, therefore, is independent of the receptor-ligand bond energy. Despite the obvious consequences of these observations for both biomaterials and cell adhesion, the physical criteria dictating the preferred failure pathways in the presence of competing mechanisms, the relative strengths of the junctions, and their relationship to the competing "bond" energies have not been established.

Recent evidence suggests that under the nonequilibrium conditions of applied external stress, the critical detachment force per bond, not the equilibrium bond energy, may determine cross-bridge strengths (Evans, 1985; Evans et al., 1988; Chirovolu et al., 1994). Thus, the weakest adhesive junction in the assembly—the smaller critical detachment force—would determine the preferred unbinding pathway. Because the pull-off force is the energy gradient of the associated detachment mechanism, the yield force per bond would be determined by both the bond energy and by the effective width of the potential well or the "bond length" (Evans, 1985; Evans et al., 1988; Chirovolu et al., 1994; Moy et al., 1994). Thus, if the weakest of the forces associated with the competing rupture mechanisms determine

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the failure loci, then the relative energy gradients along the bond axes $\vec{F}_b/\vec{F}_m = \vec{\nabla} \cdot E_b/\vec{\nabla} \cdot E_m$, not the energies alone E_b/E_m, would determine both the sites of detachment and their adhesive strengths.

The hypothesis that bond energies were not the primary determinants of the strengths of receptor-mediated bonds was first proposed to account for observed membrane failure—as opposed to receptor-ligand bond rupture—during cell detachment (Bell, 1978). In the latter case, the two dominant competing detachment mechanisms were membrane failure and receptor-ligand bond rupture. Significantly, the observed dominance of membrane failure despite the greater energy cost of pulling out membrane anchors relative to specific bond rupture was attributed to differences in the effective receptor-ligand and membrane "bond lengths." This followed because the critical detachment force along the bond axis per specific receptor-ligand bond relative to that of lipid-anchor pullout is given by

$$\frac{F_b}{F_m} = \frac{kL_b}{2\pi a_0 \gamma} = \frac{\frac{2E_b}{L_b}}{\frac{E_m}{L_m}}, \qquad (1)$$

where E_b and E_m are the bond rupture and membrane failure energies, respectively, and L_b and L_m refer to the "effective lengths" of the specific bond and hydrophobic anchor, respectively (Evans, 1985; Evans et al., 1988; Chirovolu et al., 1994). Thus, for comparable bond energies, $E_m \approx E_b$, the force ratio in Eq. 1, which indicates that the weaker bond would be determined by L_m/L_b, the ratio of bond lengths. Approximating the effective receptor bond length as 0.4 nm, the length of a hydrogen bond, and the length of a typical lipid anchor traversing half the bilayer as $L \approx 2.0$ nm, we obtain $F_b/F_m \approx 5$. In short, the greater length of the hydrophobic anchor relative to that of the specific bond would result in a smaller energy gradient, hence a weaker detachment force, for lipid anchor pullout. The resulting propensity to tear out membrane anchors during membrane unbinding, therefore, is a direct consequence of the large difference in the "bond lengths." This analysis also predicts that, because of differences in the effective "bond lengths," the crossover between the two mechanisms would occur at receptor-ligand bond energies significantly lower than the hydrophobic pullout energy, or $E_b/E_m < 1$. Consequently, in the absence of more robust or additional anchoring mechanisms such as multiple transmembrane segments or cytoskeletal attachment, the detachment of receptor-anchored cells would occur via membrane failure.

It is now generally accepted that the bond yield force, or the energy gradient, determines the adhesive strength of receptor-mediated cross-bridges. The relationship between the yield force and the equilibrium bond energy, however, has never been directly demonstrated. This work presents the first experimental demonstration 1) that the tensile strengths of specific, noncovalent cross-bridges determine the mode of cross-bridge failure, 2) that the critical detachment force is determined by both the bond energy and by the effective bond length, and 3) that the affinity at which crossover from preferential membrane rupture to bond fracture occurs is consistent with Eq. 1. In this work, we used a surface forces apparatus (Mark II) to determine the adhesive strengths and the molecular mechanisms associated with the detachment of membranes coupled by specific bonds between streptavidin and biotin or biotin-analogs on opposing membranes. Biotin analogs with affinities ranging over 10 orders of magnitude were used to establish the relationship between specific receptor-ligand bond energies, lipid pull-out energy, and the dominant mechanism of adhesive failure.

MATERIALS AND METHODS

Materials

Stearoyl-oleoyl-phosphatidyl ethanolamine (SOPE), and dipalmitoyl phosphatidyl ethanolamine (DPPE) were purchased from Avanti Polar Lipids (Alabaster, AL). Streptavidin was a gift from Boehringer-Ingelheim (Ingelheim, Germany). The ligands 2-(4′-hydroxyphenylazo)benzoic acid (HABA, $K = 2 \times 10^5$ M^{-1}), lipoic acid (LA, $K = 2 \times 10^6$ M^{-1}), desthiobiotin (DTB, $K = 10^{13}$ M^{-1}), and biotin ($K = 10^{15}$ M^{-1}) (Green, 1975) were covalently coupled via their free carboxyls to di-octadecylamine (DODA) lipid via a four ethyleneoxide unit $(EO)_4$ spacer (Müller, 1994). All salts were the highest purity grade, and all solutions were prepared from MilliQ water.

Bilayer preparations

The preparation of oriented supported streptavidin monolayers for use in direct force measurements has been described previously (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992). The streptavidin surface density in these preparations is 45 ± 5 nm^2/molecule, and there are two exposed binding sites per protein (Darst et al., 1991; Schmidt et al., 1992; Vaknin et al., 1991; Spinke, 1993). In this work, the matrix lipid in the outer leaflet of the supporting bilayer was SOPE, which remained in the fluid liquid crystalline state at 25°C, the temperature of the measurements. The lipid conjugates with biotin and biotin-analogs were incorporated at 5 mol% with the SOPE matrix lipid. A monolayer of the mixture was deposited by Langmuir-Blodgett deposition from the vapor-water interface at 0.60 ± 0.02 nm^2/lipid (12 nm^2/ligand) onto a solid tilted-crystalline monolayer of DPPE supported on mica as described previously (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992). The resulting ligand-presenting bilayer was mounted in the chamber of the apparatus opposite the streptavidin monolayer. In these systems, the biotin analogs are anchored in the membrane via the hydrocarbon tails of the DODA molecule; consequently, rupture of the receptor-ligand cross-bridges can occur either at the receptor-ligand bond and/or within the membrane interior.

Force measurements

The forces between planar bilayer membranes supporting oriented monolayers of streptavidin and opposed membranes presenting corresponding ligands were measured with a Mark II surface force apparatus. Measurements were conducted at 25°C in a bathing medium of 1 mM phosphate buffer (1 mM total phosphate) at pH 7.2, which was saturated with SOPE to prevent lipids from desorbing from the bilayers during the experiment. Solutions were filtered as described previously (Leckband et al., 1994) before they were used to fill the sample chamber of the apparatus.

The initially measured intermembrane force versus distance profile between two unperturbed streptavidin and biotin analog membranes is shown in Fig. 1 *A*. The two cylindrically curved surfaces with an undeformed geometric average radius R initially were brought into adhesive contact and then separated within 2–5 min. The *initial* adhesive force was determined from the tensile force required to detach the adherent surfaces (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992). Immediately after the surfaces were separated, the interactions were remeasured and compared with the initially measured intersurface force profiles. That lipid pull-out occurred during separation was established on the basis of detachment-induced changes in the intermembrane forces, namely, the 1) reduction in the intermembrane adhesion remeasured immediately after separation, 2) outward shifts in the "equilibrium separation" relative to D = 0 nm, and 3) changes in the intermembrane force profiles at larger separations after the initial membrane separation. In particular, the effects of resulting bilayer damage could be directly visualized in situ with the optical technique of the surface force apparatus (see below).

In these measurements, the separation between the two surfaces defined as D = 0 nm refers to the *initial* equilibrium separation between the two receptor- and ligand-coated surfaces, that is, the position of the short-range adhesive minimum between two surfaces that had not previously been in contact (Fig. 1) (Leckband et al, 1994). This position also corresponds to contact between the outer protein and bilayer surfaces, based on the determined thicknesses of the streptavidin monolayer and lipid bilayers at the end of the experiment (Leckband et al., 1994; Marra and Israelachvili, 1985).

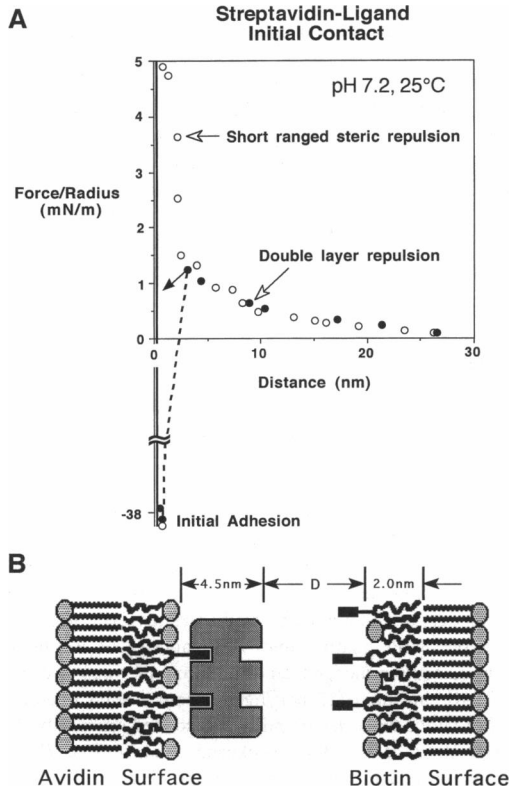

FIGURE 1 Initial forces measured between unperturbed streptavidin and ligand surfaces. (*A*) The normalized force Force/Radius F/R versus Streptavidin-ligand separation distance D for the *initial* interaction between the two surfaces at 25°C and pH 7.2 in 0.1 mM sodium phosphate buffer (0.1 mM total phosphate). The surface densities were 45 nm^2/streptavidin and 12 nm^2/biotin. The decay length of the double layer force at $D > 2$ nm is 12.5 nm (theoretical decay length λ_{th} = 15 nm). The solid arrow indicates the position from which the two surfaces jump to adhesive contact under the influence of the intersurface forces. Streptavidin-ligand contact occurred at D = 0 nm. Open and filled circles refer to separate measurements. The differences in the observed forces, namely, the steric repulsive force at $D < 2$ nm, are attributed to the time-dependent reorientations of the biotin headgroups that precede biotin-streptavidin binding. The tensile force required to detach the surfaces determines the depth of the adhesive minimum at D = 0 nm. (*B*) Molecular dimensions and proposed molecular orientations during the measurement.

RESULTS

Definition of intersurface contact

Intersurface contact, D = 0 nm, was defined by the equilibrium separation distance between the two surfaces—the location of the intersurface adhesive minimum. This corresponded to contact between the outer protein surface and the opposed bilayer surface based on the determined thickness of the samples between the mica sheets when the surfaces were in contact (Leckband et al., 1994; Marra and Israelachvili, 1985). The measured total thickness of both organic layers were determined from the change in the distance of closest intersurface approach relative to D = 0 nm that followed the destruction of the layers by UV irradiation (Leckband et al., 1994). The thickness changes determined by the latter method were 14.5 ± 0.2 nm, consistent with the determined 5.1-nm thicknesses of the planar bilayers and the crystallographically determined 4.3-nm thickness of streptavidin (Darst et al., 1991; Weber et al., 1992).

Determination of the dominant failure pathway

The tendency either to break bonds or to extract lipid anchors from the membranes was initially estimated on the basis of the relative energy costs associated with each mechanism. The calculated energy cost to pull a 2.0-nm double-chain lipid from a bilayer membrane is $2\pi a_o \gamma L$, or 30 kT (Evans, 1985; Evans et al., 1991; Chirovolu et al., 1994; Bell, 1978; Cevc and Marsh, 1987) (Table 1). Similarly, the molar free energy penalty for breaking streptavidin-ligand bonds was assumed to be equivalent to the equilibrium bond energy determined from $\Delta G = -RT \log K$, and the energy per bond $E_b = -kT \log K$ (Table 1). Based on energetic considerations alone, one would predict that preferential bond rupture would occur when $E_b/30kT < 1$. These purely energetic data in Table 1 suggest that the streptavidin-LA and streptavidin-HABA bonds would preferentially fail.

When two previously unperturbed streptavidin and biotin (or biotin analog) surfaces were initially brought into contact, the distance dependence of the measured intersurface

TABLE 1 Summary of theoretical thermodynamic parameters

Analog	K (M^{-1})	E_b/kT	E_b/E_m
Biotin	10^{15}	34	1.2
DTB	10^{13}	30	1.0
LA	2×10^6	14	0.5
HABA	$<10^5$	<12	0.4

forces was similar to that shown in Fig. 1 *A*. The long-range interactions ($D > 2$ nm) were dominated by the electrostatic double-layer repulsion between the similarly charged surfaces. At $D < 2$ nm, the specific streptavidin-ligand attraction exceeded the spring constant, and the surfaces jumped into the strong adhesive minimum (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992). The initial adhesive force of $F/R = -38 \pm 4$ mN/m, indicated by the depth of the minimum at $D = 0$ nm, was determined from the tensile force required to separate the membranes (Marra and Israelachvili, 1985; Chen et al., 1991) and was consistent with previous measurements of specific streptavidin-biotin intermembrane adhesion (Table 2) (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992). If membrane detachment occurred via the reversible rupture of the specific streptavidin-biotin bond, then the intermembrane forces remeasured after separation would be identical to the initially measured force profiles.

In contrast, cohesive failure via lipid extraction resulted in significant membrane alterations that were directly detected both visually and from changes in the intersurface forces measured immediately after membrane separation (Fig. 2 *A*). In these cases, the pull-out of hydrocarbon chain anchors pulled some streptavidin molecules to the biotin surface and vice versa (Leckband et al., 1994; Helm et al., 1991; Leckband et al., 1992) (Fig. 2 *B*), and the subsequently measured force profiles (Fig. 2 *A*) directly reflected the molecular details of the resulting membrane "damage." Namely, the adhesion at $D = 0$ nm decreased by nearly an order of magnitude from the initially measured -38 ± 4 to -4 ± 1 mN/m. Additionally, an "oscillatory force" was evident at $D < 9$ nm with two attractive minima separated by 4–5 nm, the thickness of a streptavidin monolayer. The oscillatory force was consistent with the steric interaction between the outer surfaces of proteins on opposite surfaces followed by their interdigitation and corresponding collapse of the steric repulsion at 5 nm (Leckband et al., 1994; Helm et al., 1991). The "pulled-out streptavidin" was inactivated because all binding sites were occupied by either biotin-lipid or ligand-lipid conjugates and, therefore, were incapable of further ligand binding. Consequently, the abolished short-range adhesion was also indicative of irreversible damage because of membrane failure.

Other methods have been used to determine the forces to rupture receptor-mediated cross-bridges (Evans, 1985; Evans et al., 1988; Kuo and Lauffenburger, 1993; Dembo et al., 1988; Kaplanski et al., 1993; Lee et al., 1994; Florin et al., 1994; Moy et al., 1994), but the optical technique of the surface force apparatus facilitates both the in situ detection of membrane disruption and the direct measurement of the associated adhesive force (Israelachvili, 1992). Significantly, the accuracy in determinations of both the distances and magnitudes of the membrane perturbations afforded by the optical detection method enabled the actual molecular origins of the resulting membrane alterations to be directly determined. The in situ detection of membrane failure also obviated the requirement for additional potentially perturbative analyses to determine the failure mechanisms (Evans

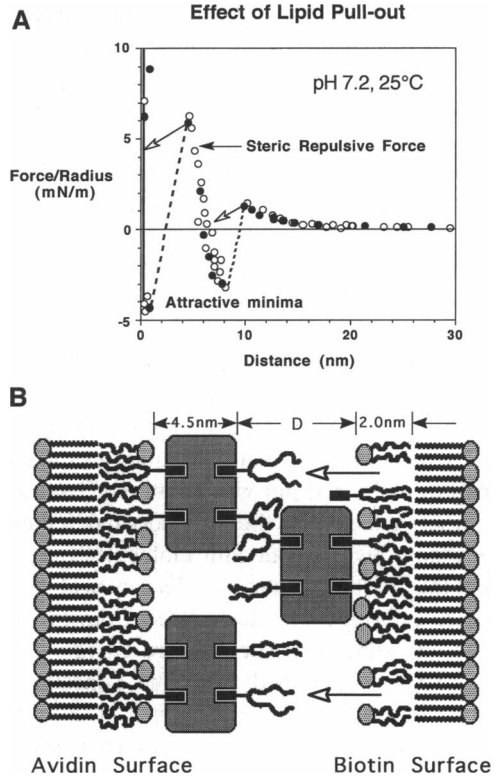

FIGURE 2 Effects of lipid pull out on intersurface forces measured subsequent to membrane detachment. (*A*) Resulting force curves measured immediately after the detachment of adhering streptavidin and biotin membranes. The decay length of the double layer force at $D > 10$ nm is 12.5 nm ($\lambda_{th} = 15$ nm). Open and filled circles refer to separate measurements. The open arrows indicate the positions at which the surfaces jump into the adhesive minima indicated. (*B*) Resulting molecular configurations due to lipid pullout.

TABLE 2 Summary of the adhesion measurements results

Analog	*F/R* (mN/m)	*E*/area (mJ/m^2)	*E*/bond (kT/bond)	F_b/F_m (Theory)	force/bond (μdyn)*
Biotin	38 ± 4	8.1	44 ± 5	6.0	8 ± 1
DTB	35 ± 5	7.4	39 ± 5	5.0	6 ± 1
LA	25 ± 4	5.3	29 ± 3	2.5	3.0 ± 0.4
HABA	8.0 ± 3	1.7	9 ± 3	<1.0	1.9 ± 0.3 (8‡)

* Assumed bond length of 2.0 nm.
‡ Assumed bond length of 0.4 nm.

et al., 1988). Consequently, in these direct force measurements, we identified the extant failure pathways by simultaneously measuring the average detachment force per cross-bridge and by directly detecting the occurrence of one of the two possible detachment mechanisms.

The data in Figs. 1 and 2 correspond to the interactions between streptavidin and biotin presenting bilayers. Bilayer damage was evident in all force versus distance profiles measured within 60 min. of membrane separation. At longer times, exchange of the "damaged" molecules in the contact area with the "undamaged" molecules in the reservoir of unaffected molecules on the surfaces results in the reannealing of the disrupted contact region as evidenced by the full recovery of the initial adhesion strength. Immediately after the initial separation, the small measured residual adhesion of -4 mN/m at $D = 0$ nm was consistent with a van der Waals attraction between the surfaces and indicated that there were no active streptavidin molecules on either surface after the initial detachment. The critical detachment force per cross-bridge was determined from the measured intermembrane adhesion. The fracture energy density per unit area ΔE was obtained from the adhesive force at contact F_c using $\Delta E = 2F_c/3\pi R$ (Chen et al., 1991). The latter energy density is the area averaged energy; consequently, although the bonds may experience different stresses throughout the contact region, these measurements reflect the average detachment force. Because the area per cross-bridge is half the estimated protein area of 45 ± 5 nm^2/molecule (2 sites/protein) (Darst et al., 1991; Schmidt et al., 1992; Vaknin et al., 1991), the calculated adhesive energy per streptavidin-biotin cross-bridge was 35 kT (Leckband et al., 1994). Reported values for streptavidin surface coverages on similar monolayers range from 67% on fluid substrates, as in this work, to 55% on amorphous substrates (Darst et al., 1991; Schmidt et al., 1992; Vaknin et al., 1991). This uncertainty as well as uncertainty in the protein dimensions (Darst et al., 1991; Schmidt et al., 1992; Vaknin et al., 1991) introduces <18% error in the critical force determination. Assuming that the predominant unbinding mechanism was lipid pullout and that the corresponding "bond length" was 2.0 nm, the average detachment force per bond was 8 ± 1 μdyn (Table 2), consistent with theoretical predictions and with previous experimental results (Evans, 1985; Evans et al., 1991); consequently, both the observed membrane damage and the calculated force per cross-bridge were consistent with membrane failure. Identical results were obtained with DTB derivatized surfaces (Table 2): namely, the initial adhesive force was -35 ± 4 mN/m, and detachment induced both membrane damage and the abolishment of the specific short-range adhesion. The calculated force per DTB cross-bridge was also similar at 6 ± 1 μdyn. Consequently, in both cases, rupture occurred preferentially within the lipid matrix, consistent with predictions based on the data in Table 1.

Despite the preferential streptavidin-LA bond rupture anticipated solely on the basis of the relative bond energies (Table 1), the postdetachment force profile shown in Fig. 3

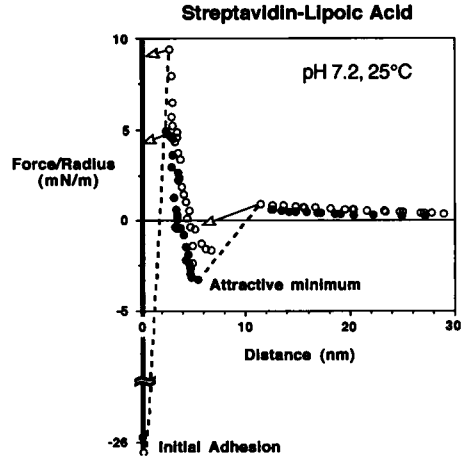

FIGURE 3 Measured forces between streptavidin and lipoic acid surfaces after membrane separation. Force profiles for the interaction between streptavidin and lipoic acid surfaces measured at 25°C and pH 7.2. The short-range minimum at $D = 0$ nm refers to the *initial* adhesion between membranes. The open arrows indicate the distances from which the surfaces jumped into adhesive contact after membrane separation. The resultant force profile indicates that the detachment of LA-streptavidin-coupled membranes still incurs significant surface damage. The open and filled circles refer to separate measurements.

evidenced substantial membrane damage during the separation. The initial adhesive force of -25 mN/m, however, was lower than observed with either the biotin or DTB membranes. Because both the streptavidin and ligand densities were identical in all experiments, and the same number of bonds presumably formed during intermembrane contact, the smaller initial adhesive force suggested that a competing fracture mechanism such as bond rupture contributed to the unbinding. That the adhesive force remeasured immediately after membrane separation decreased by only 40% to -15 ± 3 mN/m provided further support for simultaneous reversible receptor-ligand bond rupture during detachment. The residual short-range adhesion after membrane separation was attributed to specific bond formation because it was an order of magnitude greater than expected for van der Waals attraction. Clearly, the initial membrane separation resulted in membrane failure as well as in the reversible rupture of some streptavidin-LA bonds. Subsequent contacts between the membranes further decreased the magnitude of the short-range adhesion, consistent with the further pull-out of the remaining active streptavidin molecules (Leckband et al., 1994).

The average critical detachment force of streptavidin-LA cross-bridges was estimated from the calculated average bond energy and its estimated 60% fractional contribution to the unbinding mechanism. The calculated adhesive energy density between the streptavidin and LA surfaces was 5.3 mJ/m^2, and the average yield force per bond, determined by assuming a 2.0-nm hydrophobic bond length, was 6 μdyn. If, however, we assumed that the measured average 28 kT/bond was due to the pullout of 40% of the membrane anchors at 35 kT per cross-bridge and to the reversible

rupture of 60% of the specific bonds, then the bond energy corresponding to the latter pathway would be (28 kT − (35 kT × 40%))/60% = 20 kT/bond. This is slightly higher than the theoretical equilibrium bond energy of 14 kT, but not unreasonable because the true fractional contribution of each mechanism was only estimated from the residual adhesion after the initial membrane separation. Furthermore, the recruitment of receptors into the contact region during separation that has been observed with other methods (Evans, 1985; Evans et al., 1991) would increase the local cross-bridge density and reduce the average measured detachment force per bond. These data, therefore, are consistent with the simultaneous occurrence of reversible bond fracture and membrane failure.

The force profile for the interaction between streptavidin- and HABA-presenting surfaces is shown in Fig. 4. Significantly, the streptavidin and HABA surfaces could be reversibly separated without any apparent damage to the surfaces or a subsequent reduction in the adhesion at $D = 0$ nm. The latter observations indicated that the membrane detachment proceeded only via the reversible rupture of the HABA-streptavidin bonds. The measured initial adhesive force of −8 mN/m was lower than measured with the other ligands but still too large to be attributed to van der Waals or to electrostatic forces. The streptavidin affinity for HABA in these preparations likely was lower than the solution affinity of 10^5 M^{-1} because its immobilized orientation prevented optimal access to the streptavidin-binding site (Weber et al., 1992; Spinke, 1993). Nevertheless, the magnitude of the short-range adhesion was consistent with specific HABA-streptavidin binding. This was substantiated further by the abolishment of the short-range attraction after the streptavidin inactivation by the addition

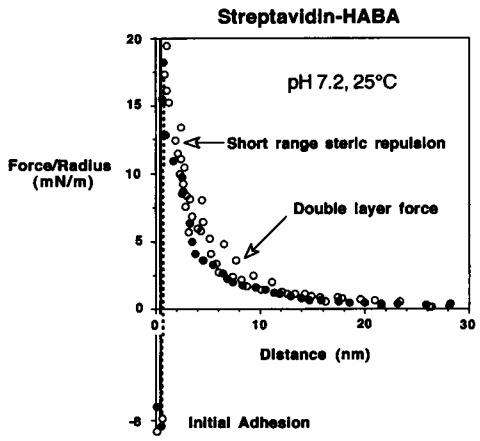

FIGURE 4 Force versus distance profile for the interaction of HABA and streptavidin surfaces. The normalized force F/R versus the distance between HABA and streptavidin surfaces after their initial separation. Forces were measured at pH 7.2 and 25°C. There was no evidence of surface damage after membrane separation, and successive compression-decompression cycles, indicated by the filled and open circles, are reproducible; consequently, the formation and rupture of cross-bridges was reversible.

of soluble biotin at 10^{-7} M (Leckband et al., 1994; Spinke, 1993).

The proposed reversible detachment by rupture of the specific bonds was confirmed further on the basis of the calculated yield force per bond: namely, the force calculated by assuming the pullout of a 2.0-nm anchor was much too low for either of the two mechanisms, but assuming a 0.4-nm bond length gave an average force of 8 μdyn, consistent with the theoretically predicted value. Thus, the absence of membrane damage, the reversibility of unbinding, and the calculated detachment force indicated that the HABA-streptavidin cross-bridges ruptured at the bond locus; hence, complete mechanistic crossover to preferential streptavidin-ligand bond rupture occurred at $K < 10^6$ M^{-1}.

DISCUSSION

The results of this study demonstrate conclusively that bond energies alone are insufficient predictors of receptor-mediated adhesion strengths. Moreover, when analyzed within the framework of previous models (Evans, 1985; Evans et al., 1991; Bell, 1978), which indicated that the detachment forces per bond govern both the detachment mechanism and the corresponding strengths of the adhesion sites, these data provide the first systematic examination of how receptor-ligand bond energies determine the mode of failure. In these measurements, both the specific receptor-ligand bonds and the lipid anchors within the membranes formed the adhesive junctions between the two opposed ligand and protein surfaces. Because membrane failure resulted in the pull-out of hydrophobic anchoring segments from the membrane into the aqueous medium, the effective "bond length" would be the length of the hydrophobic segment (Fig. 5) (Evans et al., 1991; Bell, 1978). The associated energy cost would be $E_m = 2\pi a_o L\gamma$, where a_o is the cross sectional molecular radius in the plane of the bilayer, L is the transmembrane segment length, and γ is the segment-water interfacial energy (Evans et al., 1991; Bell, 1978); consequently, the pull-out force would be $F_m = -dE_m/dL_m = 2\pi a_o\gamma$ (Fig. 5). If 26 kT are required to pull a 16-carbon (1.9-nm) double-chained lipid from a bilayer membrane (Cevc and Marsh, 1987), and the pull-out energy depends linearly on the hydrocarbon chain length L_m, then the critical lipid extraction force would be ~26 kT/1.9 nm or 6 μdyn, independent of the chain length (Evans, 1985; Evans et al., 1991). In contrast, the specific receptor-ligand bond energy is $E_b = -kT \ln K$, and the effective bond length, L_b, approximated as the effective potential well width, is expected to be ~0.4 nm, the length of a hydrogen bond (Fig. 5) (Weber et al., 1992; Lee et al., 1994; Moy et al., 1994). The approximate bond rupture force would be $F_b \approx kT \ln K/0.4$ nm (Evans, 1985; Evans et al., 1991; Chirovolu et al., 1994; Bell, 1978; Lee et al., 1994). Substituting these expressions into Eq. 1, one can readily calculate the ratio F_b/F_m for a given receptor-ligand pair and membrane anchor (i.e., as a function of bond affinity) (Table 2).

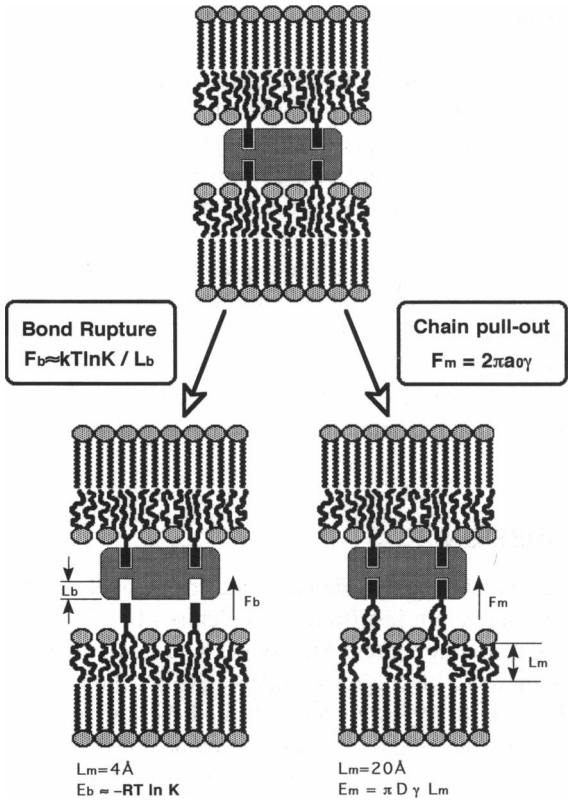

FIGURE 5 Illustration of the bond energies and effective bond lengths of the pathways mediating the detachment of receptor-coupled lipid bilayers. The specific receptor-ligand bond energy and length are given by E_b and L_b, respectively. The corresponding detachment force per bond $F_b = E_b/L_b$. The membrane "bond length," bond energy, and yield force per bond are L_m, E_m, and F_m, respectively.

The measured critical detachment forces for each of the receptor-ligand pairs most convincingly demonstrate that the detachment force per bond determines the adhesion strength. The detachment forces for biotin- or DTB-mediated adhesion were significantly lower than predicted for specific bond rupture but similar to the value expected for lipid pull-out (Evans, 1985; Evans et al., 1991; Chirovolu et al., 1994). The estimated tensile strength of the bond was slightly higher than that measured by the micropipette aspiration method, but both approaches are based on assumed cross-bridge densities. The force ratios predicted that, for this system where failure can occur either by the distraction of 2.0-nm double-chained lipids or by bond rupture, the crossover between preferential bond rupture and lipid pull-out ($F_b < F_m$) would occur for bond energies below 14 kT or at streptavidin affinities below 10^6 M^{-1}. The unbinding of streptavidin-LA-coupled membranes already signaled the simultaneous operation of both mechanisms: namely, the partially reversible detachment and an average force per cross-bridge significantly lower than required solely for lipid pullout. The completely reversible detachment of HABA-streptavidin bonds indicated complete crossover below 10^5 M^{-1}. The slight discrepancy between the predictions based on the ratios in Table 2 and the observed behavior can be attributed to the uncertainty in the streptavidin surface density and to the assumed receptor-ligand bond length—the width of the potential well. The discrepancy vanishes without altering the model predictions if instead the "effective bond length" is taken as 0.8–1.0 nm, the depth of the streptavidin-binding site.

These results directly demonstrate that the strength of receptor-mediated intermembrane adhesion is determined by the yield force per bond, that is, by the gradient of the bond energy. The bond energies at which crossover between competing failure mechanisms occur, therefore, will depend strongly on the anchoring mechanisms, the energy cost of failure, and the bond lengths. These findings also indicate that strong cell attachments could not be supported by high affinity receptors, unless the latter molecules were secured in the cell membrane by more robust linkages than membrane incorporation. This is supported by the large number of cell adhesion molecules known to form linkages with the cytoskeleton (Lauffenburger and Linderman, 1993). Presumably, the latter interactions not only play a role in transmembrane signaling, but also provide the requisite anchorage for the prevention of receptor distraction. On the basis of these and other similar findings, one might speculate further that the regulation of the latter cytoskeletal linkages is a method of controlling cell adhesion strength by simply enabling cells to switch between two different unbinding mechanisms.

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