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# RECEPTOR-CYTOSKELETAL UNBINDING in Detachment of P-selectin from PSGL-1 on Leukocytes

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*Abstract* - Using a biomembrane force probe decorated with P-selectin, discrete bonds were formed to PSGL-1 receptors on PMN surfaces and detached at speeds from ~ 1 - 100 μm/sec. High resolution tracking of the distance between probe tip and PMN revealed an initial elastic deformation that was either terminated by abrupt detachment or interrupted by yield and fluid-like extrusion of a macroscale tether plus subsequent detachment. Selecting tests that exhibited first yield then a single detachment step, we were able to quantify cohesive strengths between single PSGL-1 receptors and the PMN cytoskeleton. Prior to yield, the constant force rate was set by elastic stiffness (~ 0.25 pN/nm) of the cytostructure and the pulling speed. Collected at rates over a span from 265 pN/sec to 38000 pN/sec, distributions of yield forces were found to agree precisely with probability densities for rupture of a single bond defined by a spontaneous dissociation rate of ~ 0.5/sec and an energy barrier projected at ~ 0.25 nm along the direction of force. By comparison, single P-selectin bonds to PSGL-1 covalently attached to microspheres were slightly stronger at all loading rates as characterized by a spontaneous dissociation rate of ~ 0.15/sec and an energy barrier projected at ~ 0.22 nm. Weaker anchoring to the cytoskeleton implies frequent tether formation that can reduce the hydrodynamic load applied to selectin bonds and prolong PMN attachments to vessel walls under conditions of flow.

## I. INTRODUCTION

When leukocytes need to stick to vessel walls in the circulation, the initial step of tethering is accomplished by bonds between selectin glycoprotein and carbohydrate-like receptors and ligands resident in the membranes of both leukocytes and endothelium. Apparently designed to quickly release in the absence of a functional requirement for persistent attachment, carbohydrate:selectin bonds are weak interactions that become strong under fast detachment. However, equally important to adhesive strength, these receptors and ligands are anchored by a series of weak bonds to elements of cell cytostructure, e.g. lipid bilayer and cortical actin network. The question is to what extent cell-surface detachment involves failure of receptor-cytostructural anchoring as well as receptor-ligand unbonding, i.e. *cohesive* as well as *adhesive* failure? To address this question, we have used a biomembrane force probe BFP [1] decorated with recombinant P-selectin to test the strengths of bonds to the mucin-like P-selectin glycoprotein ligand (PSGL-1) both on neutrophils (PMN) and covalently linked to glass microbeads.

## II. METHODS

The BFP tip was prepared with a paucity of P-selectin to minimize the attachment frequency (~ 1 per 2 PMN touches and ~ 1 per 7 PSGL-1 bead touches). [As controls, few attachments were detected (~ 1/100 touches) when a P-selectin tip was touched to PSGL-1 on beads and PMNs in EDTA or when a touched by a non-specific protein tip in $Ca^{++}$.] Many cycles of approach - soft surface contact (touch) - then separation were performed by high resolution piezo displacements of a PMN or bead target. Figure 1 shows examples of force versus time under different separation speeds following attachment to PSGL-1 receptors on PMN surfaces. The force is seen to rise linearly with time up to a yield point beyond which a fluid-like extrusion process initiated formation of a macroscale tether that terminated in adhesive failure.

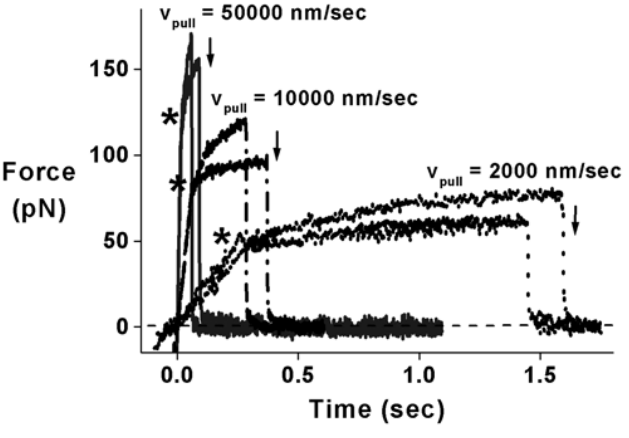


Fig.1. Force versus time during steady-speed detachment of P-selectin on the probe from PSGL-1 on a PMN surface. Asterisks mark termination of elastic deformation (yield) and onset of fluid-like extrusion to a tether. Downward arrows mark the precipitous rupture of the adhesive bond and recoil of the probe transducer.

Yield points were used to quantify strengths of receptor anchoring to the cytostructure. For comparison, force ramps in tests of P-selectin bonds to PSGL-1 on glass beads always terminated by abrupt recoil of the transducer, which provided adhesive bond strengths. With loading rates obtained from the rise in force with time, force histograms were collected at ~ 6 loading rates between 10 and 50000 pN/sec (examples in Fig. 2). The most frequent rupture forces (peaks) in distributions were used to construct the dynamic spectra of bond strength f* versus log($r_f$ = loading rate) as plotted in Fig. 3.

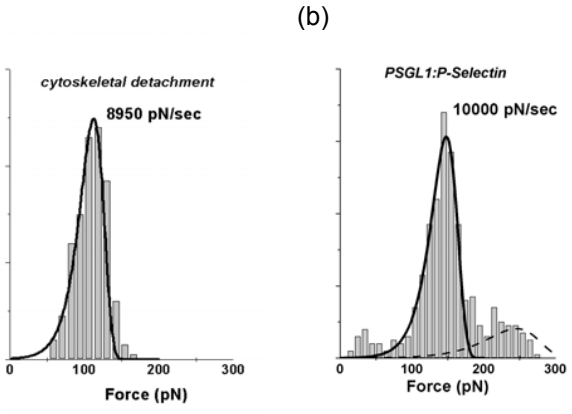


Fig. 2. Histograms of forces measured under comparable loading rates for (a) PSGL-1 detachment from the PMN cytoskeleton and (b) P-selectin detachment from PSGL-1 covalently attached to glass microspheres. Superposed are probability densities for rupture of a single bond (solid curves) with characteristics derived from the dynamic force spectra in Fig. 3. [Also shown is the probability density for rupture of two bonds (dashed curve in (b) which accounts for the few large forces that arise with the divalent FC constructs of P-selectin and PSGL-1.]

## III. RESULTS AND CONCLUSION

Dynamic spectra of forces obtained under ramps in time ($f = r_f\, t$) are simple to interpret [2] when linear regimes of force span decades in log($r_f$ = loading rate) as seen in Fig. 3. A single-sharp energy barrier governs bond strength and the log dependence on rate reflects exponentiation of the off rate under rising force, i.e. $k_{off} \approx (1/t_{off})\, \exp(f/f_\beta)$. The force scale (slope) $f_\beta = k_BT/x_\beta$ is set by thermal energy $k_BT$ and location $x_\beta$ of the barrier projected along the direction of force. The loading rate intercept $r_f^o$ yields the off rate scale, i.e. $r_f^o = f_\beta/t_{off}$. Illustrated in Fig. 2, the distributions of rupture forces at each loading rate are predicted by,

$$p(f) = (1/t_{off}\, r_f)\, \exp\{\, f/f_\beta - r_f^o\, [\exp(f/f_\beta) - 1]/r_f \,\} .$$

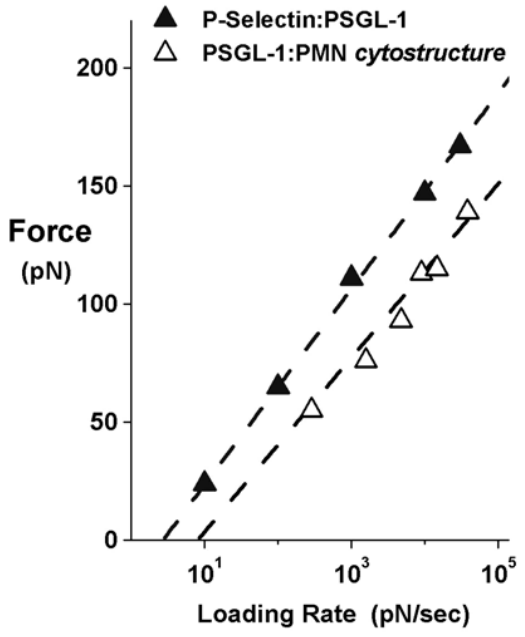


Fig. 3. Spectra of most frequent forces as functions of log(loading rate) for failure of PSGL-1:cytoskeletal anchoring (open triangles) and P-selectin:PSGL-1 molecular adhesion (closed triangles).

In Fig. 3, the results show that dynamic strengths of PSGL-1:cytoskeletal anchoring and PSGL-1:P-selectin bonds are governed by energy barriers with projected locations of $x_\beta = k_BT/f_\beta \approx 0.25$ and 0.22 nm ($f_\beta \approx 16$ and 18 pN) and spontaneous passage rates of $1/t_{off} \approx 0.5$ and 0.15 $sec^{-1}$ respectively. Hence, P-selectin bonds to PSGL-1 are somewhat stronger at all loading rates than PSGL-1 anchoring to the PMN cytostructure. Slightly weaker anchoring enables short tethers to form which reduce the hydrodynamic loads applied to selectin bonds and prolong PMN attachments under conditions of flow.

## REFERENCES

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