DEAD ZONE MEASUREMENT OF THE P-SELECTIN/PSGL-1 INTERACTION

Bryan Marshall (1), René Sánchez (1), Rodger P. McEver (2), Cheng Zhu (1)

(1) Woodruff School of Mechanical Engineering and Joint Emory/Georgia Tech Coulter School of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332-0363

(2) W. K. Warren Medical Research Institute and Department of Medicine, Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, and Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104

INTRODUCTION

Induced on activated endothelial cells and platelets, P-selectin is a cell adhesion molecule that plays a critical role in the inflammatory and thrombotic processes. P-selectin glycoprotein ligand 1 (PSGL-1) is expressed on leukocytes, and its interaction with P-selectin mediates the flowing cells tethering to and rolling on the blood vessel wall [1]. In this dynamic environment, the adhesive bonds are subject to a wide range of forces. As such, not only the kinetic rates but also how these molecules respond to mechanical stress is important to the function of this interaction.

We measured the dead zone or zero force extension of P-selectin interacting with two different ligands and an antibody. These include a dimeric P-selectin/PSGL-1 interaction [2], a monomeric sP-selectin/PSGL-1 interaction, and a P-selectin/G1 antigen/antibody interaction. The dimension of the dead zone is related to the interaction distance of the molecular pair and provides insight into the proper force versus molecular extension model for the interaction.

MATERIALS AND METHODS

P-selectin was reconstituted into a supported lipid bilayer by using the method of vesicle fusion [3]. The bilayer was formed on a glass coverslip that had been coated with PEI to reduce nonspecific adhesion. PSGL-1 or sPSGL-1, a recombinant monomeric form of PSGL-1, was coupled to a Thermomicroscopes cantilever via the capture antibody PL2 (Fig. 1).

Experiments were done by repeatedly moving the (s)PSGL-1 into and out of contact with the P-selectin. The approach speed and contact force were controlled, respectively by programming the piezo motion and using feedback from the AFM signal.

In a separate set of experiments, cantilevers were coated with G1, a monoclonal antibody that binds P-selectin near the PSGL-1 binding site. This allowed for a direct comparison between the P-selectin /PSGL-1, P-selectin/sPSGL-1 and P-selectin/G1 bonds.

The dead zone was defined as the region where a bond existed, but the applied force remained zero. This region is indicated in Fig. 2. In this region, the cantilever tip moves concurrently with the piezo as a rigid body, since no force is acting to bend the cantilever.

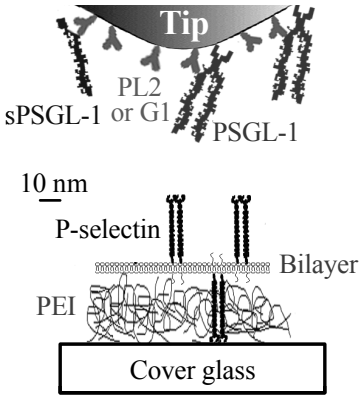

Figure 1. Experimental System

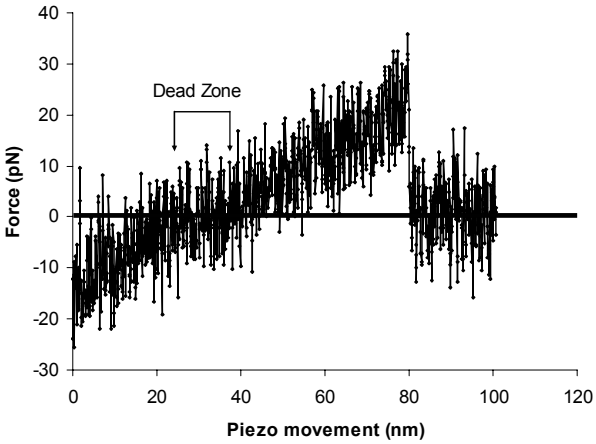

Figure 2. Dead Zone Measurement

The dead zone was measured using a program developed in MathLab to systematically analyze the fluctuating tip position as a function of time and determine the region where the slope of the force versus piezo movement was equivalent to zero (Fig. 2).

RESULTS

As expected the interaction between P-selectin and G1 resulted in the shortest dead zone measurement (Fig. 3). The contribution of the PSGL-1 molecule to the zero force extension region can be seen by comparing the G1 data with the (s)PSGL-1 data. The addition of the sPSGL-1 resulted in a doubling of the dead zone region. The dimeric bond formed in the PSGL-1 interaction reduced the dead zone region.

It is observed from the zero-force extension histogram (Fig. 4) that the G1 data is much more concentrated at the low dead zone distances, while data from both forms of PSGL-1 have multiple peaks that extend to larger distances.

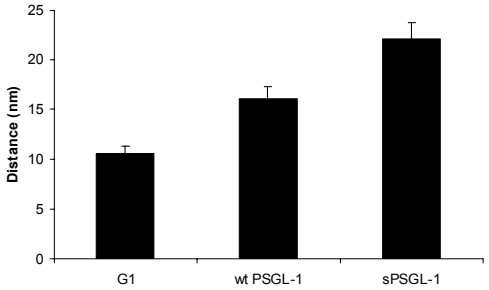

Figure 3. Dead Zone Distance

DISCUSSION

It has been suggested that the elasticity of the P-selectin/PSGL-1 complex could be represented by a modified freely jointed chain model (FJC) based on polymer mechanics [4]. According to this model, a significant part of the elasticity comes from the entropic contribution derived from the random configurations of the contorted polymer. Our data do not support the applicability of the FJC model for the P-selectin/PSGL-1 complex because the measured dead zone region is small and the force remains zero throughout. Instead, the data supports a linear relationship between force and molecular extension with the dead zone representing the portion of the force-extension curve where the molecular complex is lifted until tension develops. Additionally, P-selectin and PSGL-1 have been shown to be extended, organized molecules in solution [5, 6]. Analysis of these data suggests that P-selectin and PSGL-1 behave as semi-flexible rods, remaining relatively straight under thermal excitations [7].

In previous work we have indicated that the PSGL-1 molecule contributes little to the mechanical compliance of the molecular complex. A possibility that had not been excluded was that if this molecule was highly compliant, it could completely extend below our force detection limit. From the dead zone measurements, it appears that the PSGL-1 is not extending below our detection limit. The length of PSGL-1, approximately 50 nm, precludes it from completely extending in the 10 nm that we measure. Additional analysis has compared the flexural rigidity of P-selectin and PSGL-1 and found the two to be of the same order of magnitude [7]. These data address the above concern.

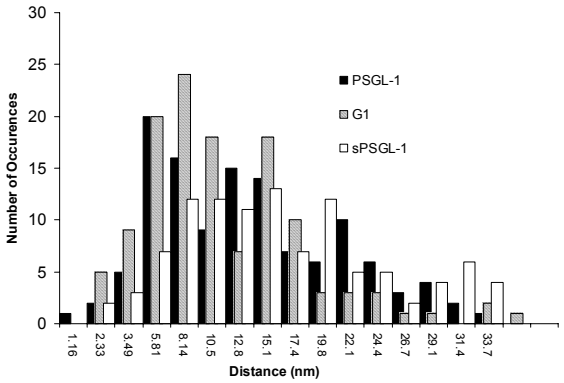

Figure 4. Dead Zone Histogram

The multiple peaks in the histogram for the PSGL-1 data indicate that there are multiple ligands on the AFM tip that may interact with the P-selectin (Fig. 4). The peak at the maximum distance is related to the maximum unstressed interaction distance between the molecules. The formation of the dimeric bond restricts this distance.

ACKNOWLEDGMENTS

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