# FLEXURAL RIGIDITY OF P-SELECTIN AND P-SELECTIN GLYCOPROTEIN LIGAND 1 

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#### Abstract

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## INTRODUCTION

P-selectin is a cell adhesion molecule expressed on activated endothelial cells and platelets. P-selectin glycoprotein ligand 1 (PSGL1 ) is expressed on leukocytes. The interaction of P-selectin and PSGL1 mediates flowing leukocytes tethering to and rolling on the vascular surface, which are initiating events in inflammatory and thrombotic processes. In this mechanically stressful environment, the P-selectin and PSGL-1 molecules are subject to a wide range of forces. As such, the flexural rigidities of these molecules and their functional implications merit investigation. Also, measuring properties of a single molecule of the size of tens of nanometers not only symbolizes a longstanding theoretical appeal but also represents quite a technical challenge. As such, the flexural rigidities of P-selectin and PSGL-1 are of interest to biophysicists. Here we report determination of these properties by analyzing the thermally excited curvature fluctuations.

## ANALYSIS

P-selectin and PSGL-1 have been visualized by electron microscopy (EM) as rod-shaped molecules [1, 2]. Their nanometer sized cross-section implies that they could be bent by applied loads as small as thermal forces. Indeed, the EM images reveal random shapes of these molecules (Fig. 1), suggesting that they underwent curvature


Figure 1 Electron photomicrographs of rotary-shadowed (A) soluble $P$-selectin ( $\mathbf{s P S}$ ), ( $B$ ) rosettes of membrane $P$-selectin (mPS), (C) a PSGL-1 rosette, and (D) single PSGL-1 molecules. Bar $=100 \mathrm{~nm}$ for both the P-selectin and PSGL-1 cases.
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Figure 2 Resting (solid curve) and fluctuating (dashed curve) shapes of sPS. A curvilinear coordinate system ( $s, \theta$ ) is set up along the resting shape. The lateral deflection $q$ is defined as shown.
fluctuations at the time the specimens were fixed. Analyzing the statistical characteristics of these random shapes may provide insights into the flexural rigidity of these molecules, as the greater the flexural rigidity, the less severely the molecules are likely bent.

Figure 2 shows the "resting shape" of sPS overlaid with a typical random shape. The resting shape was determined by averaging $\sim 40$ random shapes. First, the following rigid-body translation [by a displacement $\left.\left(y_{0}{ }^{(m)}, x_{0}{ }^{(m)}\right)^{T}\right]$ and rotation (by an angle $\alpha^{(m)}$ ) transformation was applied to the coordinates $\left(\hat{x}_{n}^{(m)}, \hat{y}_{n}^{(m)}\right)^{T}, n=1,2$, $\ldots$, measured along the mid-curve of the $m$ th molecule ( $m=1,2, \ldots$ ).

$$
\binom{x_{n}^{(m)}}{y_{n}^{(m)}}=\left(\begin{array}{cc}
\cos \alpha^{(m)} & \sin \alpha^{(m)}  \tag{1}\\
-\sin \alpha^{(m)} & \cos \alpha^{(m)}
\end{array}\right)\binom{\hat{x}_{n}^{(m)}}{\hat{y}_{n}^{(m)}}+\binom{x_{0}^{(m)}}{y_{0}^{(m)}}
$$

This aligned the molecules so that averaging of $\left(x_{n}{ }^{(m)}, y_{n}{ }^{(m)}\right)^{T}$ over $m$ resulted in the resting shape, $\left(x_{n}, \bar{y}\left(x_{n}\right)\right)^{T}$., $x_{0}{ }^{(m)}, y_{0}{ }^{(m)}$, and $\alpha^{(m)}$ are determined by minimizing the following chi square

$$
\chi_{m}^{2}\left[x_{0}^{(m)}, y_{0}^{(m)}, \alpha^{(m)}\right]=\sum_{n=1}\left[y_{n}^{(m)}\right]^{2}
$$

The lateral deflection $q_{n}{ }^{(m)}$ is quantified from the following equation:

$$
\begin{equation*}
q_{n}^{(m)}=\left[y_{n}^{(m)}-\bar{y}\left(x_{n}^{(m)}\right)\right] \Delta x_{n}^{(m)} / \Delta s_{n}^{(m)} \tag{2}
\end{equation*}
$$

where $\Delta x_{n}^{(m)}=x_{n+1}^{(m)}-x_{n}^{(m)}$ and $\Delta s_{n}^{(m)}=\left\{\left(\Delta x_{n}^{(m)}\right)^{2}+\left[\bar{y}\left(x_{n+1}^{(m)}\right)-\bar{y}\left(x_{n}^{(m)}\right)\right]^{2}\right\}^{1 / 2}$.

To analyze the lateral deflections, the molecule was modeled as an elastic beam of length $L$ and flexural rigidity $E I$. The equation of motion in the curvilinear system (cf. Fig. 1) can be written as

$$
\begin{equation*}
\rho \frac{\partial^{2}}{\partial t^{2}} q(t, s)+2 \zeta \frac{\partial}{\partial t} q(t, s)+E I \frac{\partial^{4} q}{\partial s^{4}}=f(t, s) \tag{3}
\end{equation*}
$$

where $t$ is time, $\rho$ is the density, $\zeta$ is the damping coefficient, and $f$ is the random thermal excitations. Three models were used to analyze the data, which correspond to different boundary conditions: Model $A$ assumed free ends; Model $B$ assumed two free beam segments hinged together at a single point; and Model $C$ assumed that the beam had a free end and a built-in end.

These boundary value problems were solved by expanding $q$ into series of eigenmodes $\left\{u_{i}\right\}$ with corresponding eigenvalues $\left\{\lambda_{i}\right\}$.

$$
\begin{equation*}
q(t, s)=\sum_{i} a_{i}(t) u_{i}(s) \text { where } a_{i}(t)=(1 / L) \int_{0}^{L} q(t, s) u_{i}(s) d s \tag{4}
\end{equation*}
$$

The bending energy of the beam can be written as

$$
\begin{equation*}
U=(1 / 2) E I \int_{0}^{L}\left(\partial^{2} q / \partial s^{2}\right)^{2} d s=(1 / 2) E I \sum_{i} \lambda_{i} a_{i}^{2} \tag{5}
\end{equation*}
$$

It follows from the equipartition theorem that the mean energy of each phonon equals $1 / 2 k_{B} T$ at thermodynamic equilibrium.

$$
\begin{equation*}
1 / 2 E I \lambda_{n}<a_{i}^{2}>=1 / 2 k_{B} T \text { or } E I=k_{B} T / \lambda_{n}\left\langle a_{i}^{2}>\right. \tag{6}
\end{equation*}
$$

where $k_{B}$ is Boltzmann constant and $T$ is absolute temperature.

## RESULTS AND DISCUSSION

Two soluble forms of P-selectin were analyzed. tPS is truncated after the ninth consensus repeat (CR) and asPS lacks the transmembrane domain duo to alternative RNA splicing. In the EM images, both appeared as monomers of $38 \pm 1 \mathrm{~nm}$ long with a globular domain ( $\sim 4 \mathrm{~nm}$ ) on one end and a bent $\sim 10 \mathrm{~nm}$ from the other end (Fig. 1A). The extra 8 amino acids that separate the $7^{\text {th }}$ and $8^{\text {th }}$ CRs were thought to provide more flexibility, resulting in the bent.

Analysis of the bent angle revealed a Gaussian distribution with a mean of $<\theta_{b}>=0.41$ (or $23^{\circ}$ ) and a standard deviation of $\sigma_{\theta}=0.28$ (or $16^{\circ}$ ) (Fig. 3) from the axis of the long beam segment (cf. Fig. 1). Modeling the linkage between the $7^{\text {th }}$ and $8^{\text {th }}$ CRs as an elastic hinge with an angular spring, the elastic potential energy associated with the elastic hinge is $U_{\theta}=1 / 2 k_{\theta}(\theta-\langle\theta\rangle)^{2}$. Again, the equipartition theorem requires that $\left\langle U_{\theta}>=1 / 2 k_{\theta} \sigma_{\theta}^{2}=1 / 2 k_{B} T\right.$, which yields $k_{\theta}=51 \mathrm{pN} \cdot \mathrm{nm}$.

Thus, P-selectin should be modeled as two beam segments linked by an elastic hinge with a rotational spring constant estimated from above. This analysis is currently underway. Model $A$ and $B$ correspond, respectively, setting $k_{\theta}$ to infinity and zero, thus providing the respective upper and lower bounds for the flexural rigidity. The EI values estimated using Eq. 6 are summaried in Table 1.

The membrane P-selectin (mPS) formed rosettes of 2-6 molecules by joining their hydrophobic, membrane-spanning domains (Fig. 1B). They seemed to be more curved probably because they were projecting


Figure 3 Measured (points) and fitted (solid curve) bent angle distributions of SPS. The dashed curves represent Gaussian distributions the sum of which is the solid curve.
in several directions in a rosette, which collapsed into a bent configuration as the rosette was dried and flattened. The apparent flexural rigidity estimated using Model $A$ and $C$ are also listed in Table 1. The apparent $E I$ estimated from Model $A$ is only slightly smaller, suggesting that the collapsing effect was minimal. It also suggests that the joint at the membrane-spanning domain did not significantly hinder rotation, because treating the joint as a built-in end (Model $C$ ) resulted in a 6 -fold increase in the apparent $E I$ value.

Analysis of the EM images of individual PSGL-1 molecules (54 $\pm 0.7 \mathrm{~nm}$ long) and PSGL-1 rosettes of 2-5 molecules [2] with Model $A$ yielded a substantial discrepancy in the $E I$ values (Table 1). Since rosettes only resulted in a $\sim 10 \%$ smaller $E I$ for mPS compared to sPS, the $\sim 60$-fold higher apparent $E I$ estimated from the individual PSGL-1 is likely due to the selection of a small sample of molecules with much less curved shapes. They were reported to likely be aligned by shear flow [2], which might also be straightened.

By comparison, Gittes et al. reported $E I=73,000$ and $22,000,000$ $\mathrm{pN} \cdot \mathrm{nm}^{2}$ for F -actin and microtubule, respectively [3]. These numbers explain why microtubules as long as tens of microns and F-actin as long as microns are straight, but P-selectin and PSGL-1 as short as ten some nanometers are curved.

Modeling P-selectin as a circular rod of radius of 1 nm , the moment of inertia can be calculated as $I=0.785 \mathrm{~nm}^{4}$. This allows us to calculate the Young's moduli, which are shown in Table 1. By comparison, the Young's modulus for F -actin is $620 \mathrm{pN} / \mathrm{nm}^{2}$ [3].

The results of the present study suggest that P -selectin and PSGL1 behave as semi-flexible rods. This property may enable them to remain fairly straight above the apposing cell surfaces instead of being folded, which may facilitate their interaction under physiological condition.

Table 1 Summary of Flexural Rigidities and Young's Moduli

| Molecule | \# of Specimens | Model | $\boldsymbol{E I}\left(\mathbf{p N} \cdot \mathbf{n m}^{\mathbf{2}}\right)$ | $\boldsymbol{E}\left(\mathbf{p N} / \mathbf{n m}^{2}\right)$ |
| :---: | :---: | :---: | :---: | :---: |
| tPS | 42 | $A$ | 121 | 154 |
|  |  | $B$ | 40 | 51 |
| mPS | 22 | $A$ | 124 | 158 |
|  | 72 | $B$ | 43 | 55 |
| PSGL-1 <br> rosettes |  | $C$ | 114 | 145 |
| PSGL-1 <br> individual | 6 | $A$ | 729 | 928 |
|  |  | $A$ | 61 |  |

## ACKNOWLEDGMENTS

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## REFERENCES

[1] Ushiyama, S., Laue, T. M., Moore K. L., Erickson, H. P. and McEver, R. P., 1993, "Structural and functional characterization of monomeric soluble P-selectin and comparison with membrane P-selectin," J. Biol. Chem., Vol. 268, pp. 15229-15237
[2] Li, F., Erickson, H. P., James, J. A., Moore, K. L., Cummings, R. D., and McEver, R. P., 1996, "Visualization of P-selectin glycoprotein ligand-1 as a highly extended molecule and mapping of protein epitopes for monoclonal antibodies," J. Biol. Chem., Vol. 271, pp. 6342-6348.
[3] Gittes, F., Mickey, B., Nettleton, J. and Howard, J., 1993, "Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape," J. Cell Biol., Vol. 120, pp. 923-934.

