EFFECTS OF MOLECULAR LENGTH AND ORIENTATION ON KINETICS OF P-SELECTIN/LIGAND INTERACTIONS

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INTRODUCTION

Receptor/ligand interactions are basic issues to cell adhesion, which are important to many physiological and pathological processes such as inflammatory reaction, tumor metastasis, etc. Selectin/carbohydrate ligand binding have been found to mediate the rolling of leukocytes on endothelial monolayer, the first step of inflammatory cascade, due to the fast kinetic rates. Kinetic rate and binding affinity constants are essential determinants of cell adhesion. Using a well-developed small system probabilistic model and a micropipette aspiration assay, we have previously measured the P-selectin/ligand binding on apposed surfaces (two-dimensional (2D) interaction). Here we compared the 2D kinetics of different molecular lengths and different molecular orientations binding to carbohydrate ligands expressed on a human promyelocytic leukemia cell line (HL-60), upon the hypothesis that the existence of glycocalyx layer on RBC membrane and orientation of surface-bound molecules could affect the kinetics of selectin/ligand interactions.

MATERIALS AND METHODS

Micropipette aspiration assay has been described previously. Here we developed two protocols to put the interested molecules onto RBC surfaces. In the capture protocol, anti-P-selectin monoclonal antibodies (mAbs) 1478 and S12 were coated on the surfaces of human RBCs using CrCl3 protocol. Those RBCs were then used to capture P-selectin constructs with Lec/EGF (P-Lec/EGF) and with whole extracellular domains (sPs), respectively. In the direct coating protocol, sPs was directly coated to RBCs surface. RBCs from the above protocols then interacted respectively with cultured HL-60 cells expressing carbohydrate ligands. Binding probability, $P_a$, on contact duration, $t$, of selectin/ligand interaction at the contact area, $A_c$, was measured experimentally using the micropipet technique, and the kinetic rates and binding affinity were predicted using the small system probabilistic model,

$$P_a = 1 - \exp \{-A_c m_r m_l K_a^0 [1-\exp(-k_r^0 t)]\}$$

where $K_a^0$ and $k_r^0$ are zero-force binding affinity and reverse rate, respectively, and $m_r$ and $m_l$ are the site densities of selectin and ligand respectively.

RESULT

Kinetic rates and binding affinities were predicted by fitting the experimental data for various molecular lengths and orientations. Results validated the hypothesis that the molecular length and orientation may mainly affect the forward rate, but not the reverse rate, of selectin/ligand binding (Figs. 1 and 2). We also compared the combined effects of molecular length and orientation to the selectin/ligand interactions. Surprisingly, there were no significant differences in the equilibrium binding probability if one compared the binding of directly-coated sPs to HL-60 cells with the adhering of P-Lec/EGF captured via mAb 1478 to same cells (Fig. 3). These outcomes further our understanding of selectin/ligand binding and

1. Effect of Different Molecular Lengths

Figure 1. Comparison of measurements of long (sPs; squares) and short (P-Lec/EGF; cycles) P-selectin constructs binding to HL-60 cells with the predictions (solid lines). Here $<n>/m_r = A_c m_r m_l K_a^0 [1-\exp(-k_r^0 t)] = \ln[1/(1- P_a)]/m_r$. 

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2. **Effect of Different Molecular Orientations**

![Figure 2](image1.png)

Figure 2. Comparison of measurements of captured (squares) and directly coated sPs (cycles) binding to HL-60 cells with the predictions (solid lines).

3. **Combined Effect of Molecular Lengths and Orientations**

![Figure 3](image2.png)

Figure 3. Comparison of measurements of captured P-Lec/EGF (squares) via mAb 1478 and directly coated sPs (cycles) binding to HL-60 cells with the predictions (solid lines).

Kinetic parameters were obtained by fitting the data using the probabilistic model (cf. Eq. (1) and Fig. 1 caption), and summarized in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1. Summary of kinetic parameters</th>
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<tr>
<td>Parameters</td>
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<tr>
<td>$k_0$, (s$^{-1}$)</td>
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<td>$A_mK_w$, ($\mu$m$^2$s$^{-1}$)</td>
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<td>$A_mK_w$, ($\mu$m$^2$)</td>
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**CONCLUDING REMARKS**

Experimental results indicated that the molecular length and orientation mainly affect the forward rate, instead of the reverse rate, of P-selectin/ligand binding, which not only validated our hypothesis but also provided the further understanding on the molecular mechanism of receptor/ligand interactions.

**ACKNOWLEDGMENTS**

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**REFERENCES**