QUANTIFYING THE EFFECTS OF STRUCTURAL MUTATION OF P-SELECTIN GLYCOPROTEIN LIGAND-1 ON 2D BINDINGS[1)]

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Selectins mediate rolling and tethering of leukocytes on the vessel wall by rapid formation and dissociatio of selectin–ligand bonds during inflammatory response[1]. All the selectins, L -, E-, and P-selectin, bind specifically to P-selectin glycoprotein ligand-1 (PSGL-1), a homodimeric mucin. The binding sites of PSGL-1 were identified to be tyrosine sulfate at residue 46, 48, and 51, and a core-2 O-glycan capped with sialyl Lewis x (sLe^x) at Thr-57. Mutation of tyrosine replacement changed reverse kinetic rates and mechanical properties of selectin-PSGL-1 bonds under flow [1-2]. Yet the zero -reverse kinetic rates and 2D affinities were not directly measured. And amazingly, sLe^x alone was reported to be able to support rolling of microspheres on P -selectin coated plate [3]. Therefore, functionality of PSGL-1 binding sites at amino acid level needs to be quantified further.

We used an well-established micropipette assay to measure the intrinsic reverse kinetic rates and affinitie of selectin-PSGL-1 bonds[4~7]. Anti-P-selectin monoclonal antibody S12 or anti-IgG polyclonal antibody AP113 was coated onto the human red blood cells (HRBCs) using a $CrCl_3$ protocol[4~6], which then captured soluble P-selectin or L-selectin Ig chimera. Transfected Chinese Hamster Ovary (CHO) cells stably expressing wild type or mutated PSGL-1 were digested and manipulated to adhere to HRBCs. Scatchard analysis was used t determine 3D binding affinities of ^{125}I-labeled PL-1 in solution for wild type or mutated PSGL -1 on CHO cells[8].

The binding frequency depends on contact duration and sites densit , following the receptor-ligand binding kinetics. By fitting the measured data wit a previously described small system probabilistic model[4-5], kinetic rates and binding affinities were obtained. Reverse rates of PSGL-1 mutants binding to both P - and L-selectin were slightly greater than wild type. Binding affinity for wild type was tremendously higher than those for mutants, indicating that the forward rates of mutants were significantly decreased after tyrosine replacements. However, 3D affinities of both wild type and mutants did not vary much. Weak but specific bindings between sLe^x and L- or P-selectin were observed. This furthers the understandings on selectin-ligand binding at the leve of amino acid.

References

1. Ramachandran V., Nollert M. U., Qiu H., *et al. Proc. Natl. Acad. Sci. USA.* 1999. 96:13771~13776
2. Leppänen A., Yago T.,Otto V. I., *et al. J. Biol. Chem.* 2003. 278:26391~26400
3. Rodgers S. D., Camphausen R. T., and Hammer D. A. *Biophys. J.* 2001. 81:2001~2009
4. Chesla S. E., Selvaraj P., Zhu C. *Biophys. J.* 1998. 75:1553~1572
5. Long M., Goldsmith H. L.,Tees D. F., *et al. Biophys. J.* 1999. 76:1112~1128
6. Long M., Zhao H., Huang K -S., *et al. Ann. Biomed. Engi.* 2001. 29:935~946
7. Zhu C., Long M., Chesla S. E., *et al. Ann. Biomed. Engi.* 2002. 30:305~314
8. Huang J., Chen J., Chesla S. E., *et al. J. Biol. Chem.* 2004. 279:44915~44923

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