A Micropipet Aspiration System to Measure the Kinetics of Selectin/Ligand Interactions

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Abstract

A micropipet aspiration assay was set up to measure the kinetics of selectin binding to carbohydrate ligands. Reaction rates and binding affinity were estimated from the experimental data using a small system probabilistic model. This work can be extended to analyze kinetics of receptor-ligand binding in many physiological and pathological processes, such as rolling of neutrophils on endothelium.

Keywords: Force, probabilistic model, kinetic rates, binding affinity

1. Introduction

Receptor/ligand interactions are important to many physiological and pathological processes such as inflammatory reaction, tumor metastasis, etc. For example, 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 [4]. Kinetic rate and binding affinity constants are essential determinants of cell adhesion. Not until recently has kinetic measurements become experimentally possible for receptor/ligand binding on apposed surfaces, so-called two-dimensional (2D) interaction. Using a well-developed small system probabilistic model and a micropipet aspiration assay [1-3,5], we previously measured the E-selectin/ligand binding, and compared the 2D kinetics of E-selectin binding to carbohydrate ligands expressed on a human promyelocytic leukemia cell line (HL-60) with ones expressed on a human colon adenocarcinoma cell line (Colo-205) [3]. Here we further measured the kinetics of P-selectin/ligand interaction, and compared to that of E-selectin/ligand binding.

2. Materials and Methods

Micropipet aspiration assay has been described previously. Briefly, it employs an aspirated human red blood cell (RBC) as an ultra-sensitive force transducer to detect and quantify adhesion mediated by even a single receptor/ ligand bond (Figs. 1A-D). Three different types of data were measured simultaneously, one in each of the three distinct phases of an *adhesion test cycle*: the *adhesion score* in the no-load *contact phase* (t_0,t_1), the *detachment force* in the constant-rate *retraction phase* (t_1,t_2), and the *adheison lifetime* in the constant-load *holding phase* (t_2,t_3) (Fig. 1E).

The first phase examines how likely adhesion occurs. At time t_0 the selectin-coated RBC and the ligand-expressing target cell are brought together and kept in contact for a given period of time. At the end of the contact duration t_1, the computer-driven piezoelectric translator retracts the pipette until it returns to the starting position at time t_2. An adhesion, if present at the end of the contact, will result in an elongation of the RBC upon its retraction, enabling visual detection of the occurrence of adhesion. A binary score is obtained, one if adhesion occurs and zero if not. The second phase concerns the positive adhesion event only. For this, retracting the pipette at a constant rate imposes a linearly increasing force on the selectin/ligand bond(s). If the adhesion breaks up during retraction, the force f at which detachment occurs will be measured from the RBC deflection. The third phase concerns the adhesion that survives the retraction only. If the two cells remain adherent after the pipette returns to its starting position at t_2, the RBC will be held still to keep the force constant (as set by the RBC deflection). The adhesion lifetime $\tau (= t_3 - t_2)$ at that force will be recorded by a video timer until the two cells dissociates at t_3. In this work, anti-P-selectin monoclonal antibodies (mAbs) 1478 and S12 were coated on the surfaces of human RBCs using $CrCl_3$ protocol, which are used to capture P-selectin constructs with Lec/EFG (P-Lec/EGF) and with whole extracellular domains (sPs), respectively (Fig. 1F). Capture mAbs-coated RBCs were then incubated with respective P-selectin constructs before binding to cultured HL-60 cells. 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 [1-3],

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

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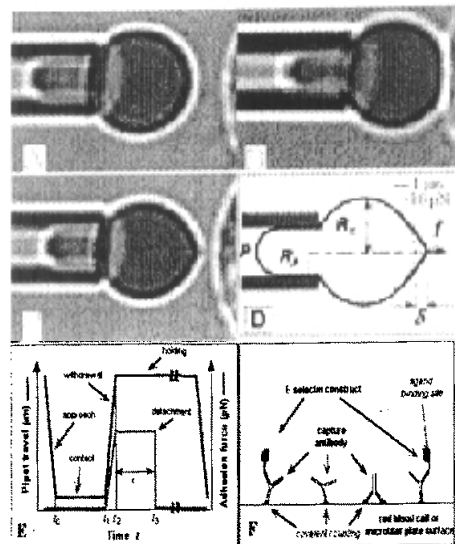

Fig. 1 *A-C*: A micropipette-aspirated RBC coated with E-selectin (left) was driven by a piezoelectric translator to make a controlled contact with the carbohydrate ligand-expressing human colon carcinoma cell (Colo-205), held stationary by another pipette (right, only partially shown). Upon the pipette withdrawal, adhesion was detected from the RBC membrane deflection. *D*: Overlay drawing of the photomicrographs shown in *A* and *C* to illustrate how the adhesion force *f* can be calculated. *E*: Schematic of the adhesion test cycle showing the pipette travel (left ordinate) and adhesion force (right ordinate) as functions of time (abscissa). *F*: Schematic of the chemistry used to coat the E-selectin construct onto the surface of RBC or of the 96-well microtiter plate.

3. Results

Control and blocking experiments were designed to address the binding specificity for P-Lec/EGF and sPs, respectively, binding to the ligands on HL-60 cells. Adhesions were abolished when the P-selectin capture mAb 1478 or S12 was replaced by an isotype-matched irrelevant mIgG1 or when the P-selectin construct was substituted by the E-selectin construct. Adhesions were completely blocked by the anti-P-selectin and anti-PSGL-1 blocking mAbs (PL1). Inclusion of EDTA (5 mM) in the medium also abrogated adhesions (data not shown). These experiments showed

that the binding was specifically mediated by P-selectin/ligand interaction.

A typical curve for dependence of binding probability on contact duration was shown in Fig. 2. Using Eq. 1, the zero-force binding affinity and reverse rate of P-selectin/ligand binding can be estimated from the measured data. These numbers were used to compare with E- and P-selectin dissociating from HL-60 cells, as well as with the data from a flow chamber assay.
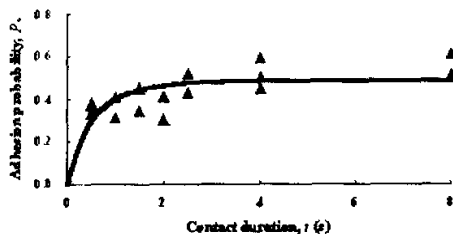

Figure 2. Comparison between measured (*points*) and predicted (*curve*) adhesion probability, P_a, on contact duration, t, for sPs binding to ligands on HL-60 cells. The theoretical prediction is based on Eq. 1. Each cell pair was used to repeat 100 cycles to estimate the P_a for that cell pair ($N = 17$).

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