Measuring very low affinity interactions between immunoglobulin superfamily cell-adhesion molecules

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Cell-adhesion molecules (CAMs) mediate multimeric interactions between cells [1]. In the immune system cellular interactions are usually transient and therefore need to be easily reversible [1]. Possible mechanisms for de-adhesion are (1) affinity down-regulation, as found with integrins [2]; (2) shedding of the CAMs, as found with CD44 [ICAM-1] [3], selectins [4], CD43 [5], and CD44 [6]; and (3) spontaneous dissociation. Spontaneous dissociation would require that CAM complexes have fast dissociation rates and low affinities. However very few affinity analyses and no kinetic analyses have been performed on CAM interactions probably because measuring low affinity protein interactions is technically very difficult.

Rat CD2 is the first CAM for which the structure of the entire extracellular portion has been determined [7]. We have recently reported that rat CD48 is a ligand for rat CD2 [3]. A preliminary estimate of the affinity of this interaction indicated that it was very low [8]. In order to overcome the difficulties associated with the analysis of low-affinity protein interactions we elected to use a BIAcore™ biosensor [9] for a comprehensive affinity and kinetic analysis. This method employs an optical system to detect refractive index changes in a dextran matrix. One ligand is covalently coupled to the matrix and the other ligand is injected over the matrix. If the injected soluble ligand binds the immobilized ligand there is an increase in the protein concentration (and therefore refractive index) in the matrix which can be detected. A major advantage of this approach is that binding is monitored in real time which enables easy equilibrium analysis and kinetic analysis of the interaction.

Figure 1. Stored sCD48-CD4 (0.6 mg/ml, aggregates) and size-fractionated sCD48-CD4 (0.6 mg/ml, no aggregates) were injected in HBS for 15 s over dextran matrix with nothing (Control) or with rat sCD2 (CD2) immobilized. HBS contains (mM): NaCl 150, CaCl2 1, MgCl2 1, Na Azide 0.01%, Hepes 25 (pH 7.5), and Sulfactant 0.005% (w/vol). All experiments performed at 37°C at flow-rate of 20 µl/min.

When two preparations of a chimeric soluble form of CD48 fused to domains 3 and 4 of rat CD4 (sCD48-CD4) [8] were injected over empty dextran matrix (Fig. 1, Control) there was a small response (measured in response units or RU). This is due to the effect of high concentrations of protein on the refractive index and similar signals are seen with all proteins (not shown). Injection of the same samples over dextran matrix to which soluble rat CD2 (sCD2) [8] was covalently coupled [9], led to a dramatically increased response (Fig. 1, CD2). Specific binding is calculated as the difference in response seen with injection through control- and sCD2-matrix. When a stored preparation of sCD48-CD4 was injected some of the binding dissociated very slowly (Fig. 1, CD2, aggregates). Gel filtration of this sample indicated that this slowly-dissociating sCD48-CD4 comprised multimeric aggregates of sCD48-CD4 present at < 2% of total protein. A sCD48-CD4 preparation used immediately after gel filtration contained slowly-dissociating material, indicating that it was free of multimeric aggregates (Fig. 1, CD2, no aggregates). Multimeric aggregates also formed in stored preparations of human and rat CD2 suggesting that aggregation may be a common phenomenon with IgSF proteins.

The affinity of the sCD2 - sCD48-CD4 interaction was determined by measuring the equilibrium binding levels when increasing concentrations of sCD48-CD4 are injected over immobilized sCD2 (Fig. 2A). Saturating binding was observed with a Kd of 90 pM. The kinetics of rat sCD2 binding to sCD48-CD4 were too fast for precise measurement but it could be established that the off-rate constant is at least 1 x 10⁻⁴ s⁻¹ and the on-rate constant is greater than 10⁷ M⁻¹ s⁻¹. CD2, CD48 and the human CD2 ligand CD58 (LFA-3) are structurally related molecules which belong to a subset of the immunoglobulin superfamily [10] and multiple interactions between members of this subset have been reported. For example, human CD2 binds human and sheep CD58 [11], as well as human CD48 [11] whereas rat CD2 binds rat [8] and mouse CD48 (PA van der Merwe, unpublished). Furthermore rat sCD2 forms a dimer in its crystal structure in which two CD2 molecules interact through their putative ligand binding sites [7]. These findings, taken together with the fact that the genes for CD2, CD48, and CD58 lie close together on human chromosome 1 [12], suggest that these molecules evolved fairly recently from an ancestral protein capable of homotypic interaction. We, therefore examined whether rat CD42 and rat CD2 were capable of homotypic interactions. No homotypic binding was detected with either sCD2 (Fig. 2B) or sCD48-CD4 (Fig. 2C) at concentrations of 40 µM and 20 µM respectively. Under the same conditions low-affinity binding of sCD48-CD4 to immobilized sCD2 (Fig. 2B) and sCD2 to immobilized sCD48-CD4 (Fig. 2C) was easily detectable. Therefore neither rat CD2 nor rat CD48 appear to bind homotypically.

In conclusion, these results provide the first detailed analysis of the kinetics and affinity of soluble, monomeric CAM interactions and suggest that binding between CAMs may be even weaker than anticipated.


Figure 2. (A) sCD48-CD4 was injected over dextran with nothing (squares) or sCD2 (circles) immobilized. Inset, Scatchard plot of specific (triangles) binding. (B) Bovine serum albumin (BSA), sCD2, and sCD48-CD4 at 1 mg/ml were injected over matrix with collagen (open) or sCD2 (filled) immobilized. (C) As in (B) but with nothing (open) or sCD48-CD4 (filled) immobilized. Conditions as in Figure 1.