Estimation of the dissociation constant of the cell adhesion molecules srCD2 and srCD48 using analytical ultracentrifugation.

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Leucocytes are cells involved in the immune response. They interact with cells and with the extracellular matrix through proteins termed cell adhesion molecules (CAMs). Rat CD2 and its counter receptor CD48 are CAMs and both are members of the immunoglobulin superfamily. They are found on the surfaces of T-lymphocytes and their heterophilic adhesion interaction mediates the adhesion of T cells to other cells such as cytotoxic targets or antigen presenting cells. The equivalent interaction in humans is between CD2 and CD58. These molecules are highly glycosylated (up to a level of 30% (w/w)) and both have molar masses of around 50,000 g/mol. The extracellular domains of the CAMs, denoted by the prefix 's', consist of two anti-parallel β-sheet domains and are believed to interact end-to-end via their N-terminal domains [1]. (The prefix 'r' is used to denote rat antigen).

The rates and affinities of the interaction have been determined by surface plasmon resonance using a BIAcore™ biosensor [2]. This involves the immobilisation of one of the molecules onto a carboxylated dextran matrix which lies on a sensor surface. The other molecule is then injected over the surface. Changes in the refractive index and hence surface plasmon resonance (spr) are then optically monitored. The dissociation constant (K_d) estimated for the srCD2-srCD48 interaction is 60-90 μM which shows that CAMs bind with a very low affinity.

srCD2 and srCD48 have both been characterised separately by sedimentation equilibrium and velocity using the Beckman XL-A analytical ultracentrifuge. srCD2 shows some self association at 5°C whilst srCD48 seems to show no self association but shows classical thermodynamic non-ideality. The association of srCD2-srCD48 has been observed as a change in apparent whole-cell weight average molar mass ($M_{w,app}$) with glycoprotein loading concentration. $M_{w,app}$ increases steadily with increasing concentration until about 7 mg/ml of each protein where a plateau occurs and maximum association is seen. From these data the mole fraction of srCD48 and srCD2 associated was determined and was used to generate an optimum association constant (and hence Kd). The srCD2-srCD48 interaction was approximated to a monomer-dimer interaction and a second order kinetics equation was used as a model for the system in a non-linear least squares curve-fitting package. The value generated is in the 100-200 μM region but requires further refinement as discussed below.

The concentration dependence of the sedimentation coefficient (as measured via sedimentation velocity) for the interaction of srCD2-srCD48 is positive (in contrast to the negative dependence normally seen in a single ideal species.) This is indicative of an associating system and supports the data generated by sedimentation equilibrium. From these velocity data the dissociation constant was also estimated. This was done using a software package (SA-Plot) based on the theory of Gilbert & Gilbert [3]. The concentration of monomer and dimer at any given total concentration can be calculated from the degree of dissociation (α) as shown in equation 1.

$$\alpha = \frac{-K_d c + \left(K_d c M_1\right)^2 + 8c^2 K_d c M_1}{4c^2} \qquad (1)$$

where K_d is the dissociation constant, c is the total concentration and M_1 is the monomer molar mass. The concentration of the monomer (c_m) and dimer (c_d) can then be found from equations 2 and 3 as follows.

$$c_m = \alpha \times c \qquad (2)$$

$$c_d = (1-\alpha) \times c \qquad (3)$$

Using these values, sedimentation coefficients can be calculated using equations 4 and 5.

$$s^c = s^0\{1 - g(c)\} \qquad (4)$$

Where s^c is the sedimentation coefficient at finite solute concentration c (corrected to standard conditions, via $s_{20,w}$), s^0 is the sedimentation coefficient at infinite dilution and g is a function of concentration [4].

$$g(c) = \frac{k_s c - \left[(c\bar{v}_s)^2\left(2\phi_p - 1\right)\right] \Big/ \phi_p^2}{k_s c - 2c\bar{v}_s + 1} \qquad (5)$$

k_s is the concentration dependence coefficient (ml/g), $\bar{v}_s$ is the swollen partial volume (ml/g) and ϕ_p is the maximum packing fraction (by volume).

SA-Plot utilises the above equations and calculates the sum of the square of residuals between experimental data and calculated curves for assumed K_d values until an optimal fit is found. The dissociation constant for the srCD2-srCD48 interaction is in the 100 μM range.

The dissociation constants generated by the two analytical ultracentrifuge techniques are comparable to each other but differ from that determined by surface plasmon resonance. This is due to the different environments of the experiments. Firstly, the immobilisation of one species in the spr experiments will affect the diffusion of this species whereas both components are freely diffusable in the sedimentation experiments. Self association has yet to be accounted for when determining the K_d values reported here. This will be accomplished in a more thorough analysis of the data. Secondly the spr and analytical ultracentrifuge data were collected at two different temperatures: spr measurements were done at 20°C whist sedimentation analysis took place in a 5°C environment.

All three data sets show that srCD2 and srCD48 have a very low affinity interaction. This is not unexpected: the surface of a cell is coated in numerous CAMs which participate in multimeric interactions. Fast off-rates are essential for spontaneous de-adhesion of cells.

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