Supporting Text

Methods

Analysis of binding data

Equilibrium-binding data were used to calculate apparent binding constants for association of peptide fragments to spectrin in the intact ghost. Writing $\overline{A}$ and $\overline{B}$ for the total concentrations of the α-peptide and membrane-associated spectrin (in terms of dimers), c for that of the αβ complexes, and K_a for the association constant:

$$c^2 - c\left(\overline{A} + \overline{B} + \frac{1}{K_a}\right) + \overline{A}\overline{B} = 0$$

This equation was used to extract the least-squares best fit for the association constant.

The rate of formation of the complex, *c*, of the α- and β-peptides is given by

$$\frac{dc}{dt} = k_+\left(\overline{A} - c\right)\left(\overline{B} - c\right) - \frac{k_+ c}{K_a}$$

where k_+ is the forward second-order rate constant, K_a the association constant, *c* the concentration of αβ complex at time *t*, and $\overline{A}$ and $\overline{B}$ the total concentrations of the two peptides. If, as here, the association constant is so low that the peptide is in large excess over endogenous spectrin, *A* >> *c*, and the kinetics reduced to a pseudofirst-order process, integration, keeping in mind that *c* = 0 when *t* = 0, then yields:

$$k_+ t = \frac{\overline{A}K_a}{\overline{A}K_a + 1} \ln \frac{\overline{A}K_a\overline{B}}{\overline{A}K_a\overline{B} - c\left(\overline{A}K_a + 1\right)}$$

The second- and pseudofirst-order rate constants, k_+' and k_+ are then related by $k_+' = k_+/\overline{A}$.

In experiments to measure dissociation rates, the inverse integrated rate equations were used to fit the data. Thus, if c is the concentration of the αβ complex on beads bearing coupled β-chain fragments, initially (at $t = 0$) c_0, whereas A and B are the concentrations of free α- and β-chain fragments, with an initial concentration of unoccupied β-chains of B_0,

$$-\frac{dc}{dt} = k_{-}c - k_{-}K_a AB$$

where k_- is the first-order dissociation rate constant. Integration then yields:

$$k_{-}t = Q^{-\frac{1}{2}} \ln\left(\frac{2K_a A + 1 + K_a B + Q^{\frac{1}{2}}}{2K_a A + 1 + K_a B - Q^{\frac{1}{2}}} \cdot \frac{1 + K_a B - Q^{\frac{1}{2}}}{1 + K_a B + Q^{\frac{1}{2}}} \right)$$

where $Q = 4K_a c_0 + (1 + K_a B_0)^2$