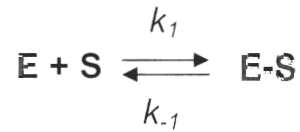


DERIVATION OF EQUATIONS FOR EQUILIBRIUM BINDING



Definitions

Dissociation constant

$$K_d = [E][S]/[ES] = k_{-1}/k_1$$

Association constant

$$K_a = [ES]/[E][S] = k_1/k_{-1}$$

Mass balance equations

$$[E]_0 = [E] + [ES]$$

$$[S]_0 = [S] + [ES]$$

K_d has units of concentration

k_{-1} is a first order rate constant with units of s^{-1}

k_1 is a second order rate constant with units of $M^{-1}s^{-1}$

Fraction of sites occupied:

$$\text{fraction} = \theta = [ES]/[E]_0 = [ES]/([E] + [ES])$$

$$\theta = \frac{[ES]}{[E]_0} = \frac{[ES]}{[E] + [ES]}$$

$$\theta = \frac{K_a[E][S]}{[E] + K_a[E][S]} = \frac{K_a[S]}{1 + K_a[S]} = \frac{[S]}{1/K_a + [S]}$$

$$\theta = \frac{[S]}{K_d + [S]}$$

If $[E] \ll K_d$ then one can assume that $[S] \cong [S]_0$ because S is not depleted due to binding to E.

$$\theta = \frac{[S]_0}{K_d + [S]_0}$$

☞ This is the equation for a hyperbola.
At $[S]_0 = K_d$ the fraction of sites bound is 0.5.

Linearized forms of the equation:

Double reciprocal plot:

$$1/\theta = \frac{[S] + K_d}{[S]} = 1 + \frac{K_d}{[S]}$$

Or for multiple sites:

$$1/\nu = \frac{[S] + K_d}{[S]} = n + \frac{K_d}{[S]}$$

Skatchard Plot

$$\theta = 1 - \frac{\theta K_d}{[S]}$$

Or for multiple sites:

$$\theta = n - \frac{\nu K_d}{[S]}$$

$$\nu = \frac{\text{moles bound}}{\text{mole E}} = n\theta$$

when you cannot assume $[S]_0 \sim [S]$
but $[S]_0 = [S] + [ES]$

Derivation of quadratic equation--with no assumptions concerning substrate concentration.

fraction = $\theta = [ES]/[E]_0 = [ES]/([E] + [ES])$ The derivation starts the same as above

$$\theta = \frac{K_d[E][S]}{[E] + K_d[E][S]} = \frac{K_d[S]}{1 + K_d[S]} = \frac{[S]}{1/K_d + [S]}$$

Fraction of sites bound relative to [S]

$$\theta = \frac{[S]}{K_d + [S]} = \frac{[S]_0 - [ES]}{K_d + [S]_0 - [ES]} = \frac{[ES]}{[E]_0}$$

Substitution of $[S] = [S]_0 - [ES]$

$$[ES](K_d + [S]_0) - [ES]^2 = [E]_0[S]_0 - [ES][E]_0$$

$$[ES]^2 - [ES](K_d + [S]_0 + [E]_0) + [E]_0[S]_0 = 0$$

Form of equation require solution as the roots of the quadratic equation

Solution is quadratic equation:

$$ax^2 + bx + c = 0$$

solution provided by the roots of the quadratic

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$K_d = \frac{[E][S]}{[ES]}$$

$$[ES] = \frac{(E_0 + S_0 + K_d) - \sqrt{(E_0 + S_0 + K_d)^2 - 4 \cdot E_0 \cdot S_0}}{2}$$

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$$\theta = \frac{(E_0 + S_0 + K_d) - \sqrt{(E_0 + S_0 + K_d)^2 - 4 \cdot E_0 \cdot S_0}}{2 \cdot E_0}$$

This equation must be used rather than the hyperbola whenever the enzyme concentration is comparable or greater than the dissociation constant. As a general rule, if $[E]_0$ is less than 5 times the K_d , the hyperbolic fit is probably adequate.

Fluorescence data

$$F = F_0 + \Delta F \cdot \theta$$

$$\text{where } \Delta F = F_\infty - F_0$$

and θ is defined by either:

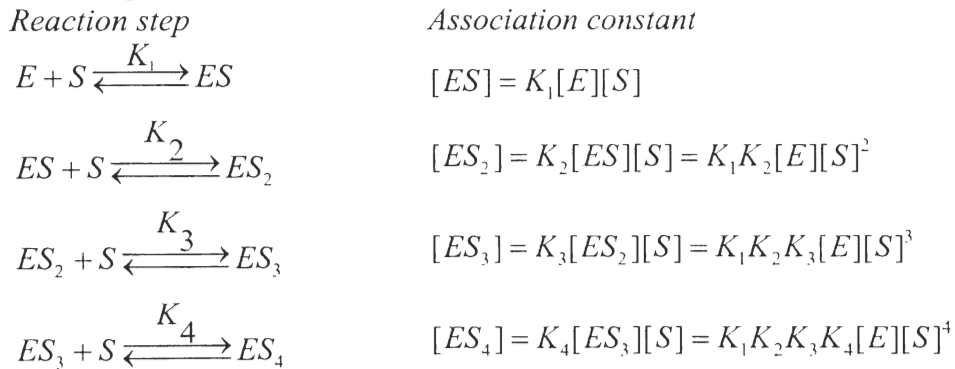
$$\theta = \frac{[S]_0}{K_d + [S]_0} \quad \text{OR.....}$$

This equation normalizes the observable signal to a scale that can be related to fractional occupancy, θ .

$$\theta = \frac{(E_0 + S_0 + K_d) - \sqrt{(E_0 + S_0 + K_d)^2 - 4 \cdot E_0 \cdot S_0}}{2 \cdot E_0}$$

The same equations could be applied to any signal, such as counts per minute or other measure of bound substrate.

Multiple Binding Sites:



Solution of fraction of sites occupied for a two-step binding sequence.

Mass balance equations:

$$[E]_0 = [E] + [ES] + [ES_2]$$

$$[S]_0 \approx [S] \text{ (negligible amount bound)}$$

Fraction of sites bound:

$$\theta = ([ES_1] + [ES_2]) / [E]_0$$

$$= \frac{[ES_1] + [ES_2]}{[E] + [ES_1] + [ES_2]}$$

Substitution of bound states:

$$[ES_1] = K_1[E][S]$$

$$[ES_2] = K_2[ES_1][S] = K_1K_2[E][S]^2$$

$$\theta = \frac{K_1[E][S] + K_1K_2[E][S]^2}{[E] + K_1[E][S] + K_1K_2[E][S]^2}$$

$$\theta = \frac{K_1[S] + K_1K_2[S]^2}{1 + K_1[S] + K_1K_2[S]^2}$$

Fraction of sites bound

If the equations are defined for moles of substrate bound per mole of dimers instead of per mole of active sites, the equations becomes:

$$v = \frac{K_1[S] + 2K_1K_2[S]^2}{1 + K_1[S] + K_1K_2[S]^2} \quad \text{where } v = \frac{\text{moles S bound}}{\text{mole of dimers}}$$

Accordingly, the binding equation ranges from 0-2 moles bound rather than from 0-1 fraction of sites occupied.