

Derivation of Case 4

Let us attempt to write down one or more solutions of the fourth and final case of protection, in which the inactivator is subject to decay and the protecting agent gives partial protection.

$$d\{[E-I] + [E-I\bullet P]\}/dt = k_i[E][I] + k_{ip}[E\bullet P][I]$$

$$[E\bullet P] = [E][P]/K_p$$

$$d\{[E-I] + [E-I\bullet P]\}/dt = k_i[E][I] + k_{ip}[I][E][P]/K_p$$

$$d\{[E-I] + [E-I\bullet P]\}/dt = [E][I]\{k_i + k_{ip}[P]/K_p\}$$

$$[E_{tot}] = [E] + [E\bullet P] + [E-I] + [E-I\bullet P]$$

$$[E-I] + [E-I\bullet P] = [E_{tot}] - \{[E] + [E\bullet P]\}$$

$$[E-I] + [E-I\bullet P] = [E_{tot}] - \{[E] + [E][P]/K_p\}$$

$$[E-I] + [E-I\bullet P] = [E_{tot}] - [E]\{1 + [P]/K_p\}$$

$$[E]\{1 + [P]/K_p\} = [E_{tot}] - ([E-I] + [E-I\bullet P])$$

$$[E] = \{[E_{tot}] - ([E-I] + [E-I\bullet P])\} / (1 + [P]/K_p)$$

Repeating from above,

$$d\{[E-I] + [E-I\bullet P]\}/dt = [E][I]\{k_i + k_{ip}[P]/K_p\}$$

Substituting for [E]

$$d\{[E-I] + [E-I\bullet P]\}/dt = \{[E_{tot}] - ([E-I] + [E-I\bullet P])\} / (1 + [P]/K_p) [I]\{k_i + k_{ip}[P]/K_p\}$$

Replacing [I] with a function that describes its decay,

$$d\{[E-I] + [E-I\bullet P]\}/dt = \{[E_{tot}] - ([E-I] + [E-I\bullet P])\} / (1 + [P]/K_p) \{k_i + k_{ip}[P]/K_p\} [I_0] \exp(-k't)$$

$$d\{[E-I] + [E-I\bullet P]\} / \{[E_{tot}] - ([E-I] + [E-I\bullet P])\} = \{(k_i + k_{ip}[P]/K_p) / (1 + [P]/K_p)\} [I_0] \exp(-k't) dt$$

Let $u = \{[E-I] + [E-I\bullet P]\}$, $a = [E_{tot}]$, and $b = -1$

$$\int \frac{du}{a + bu} = (1/b) \ln(a + bu)$$

$$\int_0^u \frac{du}{a+bu} = (1/b) \ln \frac{(a+bu)}{a}$$

$$\int_0^t \exp(-k't) dt = (-1/k') \{ \exp(-k't) - \exp(0) \} = (1/k') \{ 1 - \exp(-k't) \}$$

Using this information, let us integrate the lefthand side of the previous equation from zero to $([E-I] + [E-I \bullet P])$ and the righthand side from zero to t .

$$\int_0^{([E-I]+[E-I \bullet P])} \frac{d([E-I]+[E-I \bullet P])}{[E_{tot}] - ([E-I]+[E-I \bullet P])} = \int_0^t \left(\frac{k_i + k_{ip}[P]/K_p}{1+[P]/K_p} \right) [I_0] \exp(-k't) dt$$

We can write the left side of the integrated equation in a number of ways:

$$(-1) \ln \{ ([E_{tot}] - ([E-I] + [E-I \bullet P])) / [E_{tot}] \} = (-1) \ln \{ ([E] + [E \bullet P]) / [E_{tot}] \} = (-1) \ln(1 - \sigma)$$

The term $(1 - \sigma)$ is the fraction of enzyme remaining in the active form at any time.

Equating the lefthand and righthand sides of the integrated equation and moving (-1)

$$\ln(1 - \sigma) = (-1)[I_0](1/k') \left(\frac{(k_i + [P]/K_p)}{(1+[P]/K_p)} \right) (1 - \exp(-k't))$$

$$\ln(1 - \sigma) - (-1/k')[I_0] \left(\frac{k_i + k_{ip}([P]/K_p)}{(1+[P]/K_p)} \right) (1 - \exp(-k't))$$

We can define k_{app} in a similar way to what we did before:

$$k_{app} = \left(\frac{(k_i + k_{ip}[P]/K_p)}{(1+[P]/K_p)} \right)$$

We can rewrite the equation above as

$$\ln(1 - \sigma) = (-1)[I_0](k_{app}/k')(1 - \exp(-k't))$$

The fraction of remaining enzyme activity is a function of the concentration of P and of the rate of decay of the inactivator.

Analogy to explain the behavior of a reversibly bound protective agent

Consider the following analogy: Suppose that two water reservoirs are connected by a pipe, and one of the reservoirs has a slow leak. The water levels in the two tanks will decrease essentially equally as long as the flow between them is fast enough to equilibrate their levels. This is essentially what happens when using a gradient maker in ion exchange chromatography. The reservoir without the leak is EP (see below for the meanings of symbols), and the reservoir with the leak is E. The leak is the conversion of E to E-I. One can also imagine that the two tanks do not have to be the same volume, which corresponds to values of [E] and [EP] which are not equal to each other. The ratio of [E] to [EP] depends on the values of [P] and K_p .

Let us use the following symbols

E	enzyme
I	an irreversible inactivator
I'	an inert form of the inactivator, one that is unable to react with E
E-I	inactive enzyme
P	a reversibly bound protective agent
K_p	the dissociation constant between the enzyme and P
k_i	the second order inactivation rate constant between E and I
k_{iP}	the second order inactivation rate constant between EP and I
k_v	the pseudo-first order rate constant = $k_i[I]$
k_{\bullet}	the pseudo-first order rate constant = $k_{iP}[I]$
k'	the first-order rate constant for breakdown of I to I'
k_{obs}	the pseudo-first order rate constant governing the apparent loss of activity \pm P.
k_{app}	the second order rate constant governing the apparent loss of activity \pm P.
$\exp(x) e^x$	
σ	fraction of enzyme in the irreversibly inactivated form: $([E-I] + [E-I\bullet P])/[E_0]$
$1 - \sigma$	fraction of enzyme in the active form: $([E] + [E\bullet P])/[E_0]$

Notes

When t is used as a subscript, it means time (t may be implicit). When tot is used as a subscript, it means total. When a 0 is used as a subscript, it means $t = 0$. Reversibly bound enzyme complexes may be symbolized as either EP or $E\bullet P$. Bracketed quantities, such as [E], are concentrations. [E], [E-I], $[E\bullet P]$, and $[E-I\bullet P]$ are functions of time; [I] may be a function of time.