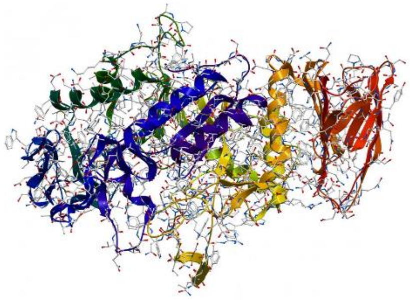


CEN-CHE 422
ENZYME ENGINEERING

Substrate Inhibition

Sometimes when a large amount of substrate is present, the rate of enzyme-catalyzed reaction is decreased by the excess substrate. This phenomenon is called *substrate inhibition*.

The excess of substrate inhibits the enzyme.

In such reactions, substrate concentrations higher than $C_S=(C_S)_{max}$, which give the $r=r_{max}$ value, are not studied.

The quantitative relationship between the substrate concentration and the reaction rate for substrate-inhibited reactions can be modeled using Michaelis-Menten approach.

Reaction steps:

$$E + S \leftrightarrow ES \qquad k_1, k_{-1}$$

$$ES + S \leftrightarrow ESS \qquad k_i, k_{-i}$$

K_i = inhibition constant

$$ES \rightarrow E + P \qquad k_2$$

$$r = \frac{r_{max} C_s}{K_m + C_s\left(1 + \frac{C_s}{K_i}\right)}$$

MM type equation for substrate inhibition

Similar to noncompetitive inhibition model

If MM equation is linearized (LB):

$$\frac{1}{r} = \frac{K_m}{r_{max}}\frac{1}{C_s} + \frac{1}{r_{max}}\left(1 + \frac{C_s}{K_i}\right)$$

At small values of $C_{S;}$ this equation is similar to the MM equation.

Thus, the intercept of the line drawn in the LB plot at high $1/C_S$ values gives the value $1/r_{max}$ and the slope gives the value of K_m/r_{max}.

to find $(1/C_s)_{min}$:

$$\frac{d(1/r)}{d(1/C_s)} = 0$$

$$\left(\frac{1}{C_S}\right)_{min} = \frac{1}{\sqrt{K_m K_i}}$$

$$(C_S)_{max} = \sqrt{K_m K_i}$$

K_i is found from $(1/C_s)_{min}$ or $(C_S)_{max}$

In the absence of inhibitor, the MM equation is integrated as follows:

$$r = -\frac{dC_S}{dt} = \frac{r_{max} C_S}{K_m + C_S}$$

$$r_{max}\, dt = -\frac{K_m + C_S}{C_S} dC_S$$

$$r_{max} \int_0^t dt = -\int_{C_{S_0}}^{C_S} \frac{K_m + C_S}{C_S} dC_S$$

$$r_{max}\, t = -K_m \int_{C_{S_0}}^{C_S} \frac{dC_S}{C_S} - \int_{C_{S_0}}^{C_S} dC_S$$

$$r_{max} t = -K_m \ln \frac{C_S}{C_{S_0}} - \left(C_S - C_{S_0}\right)$$

$$r_{max} t = K_m \ln \frac{C_{S_0}}{C_S} + \left(C_{S_0} - C_S\right)$$

This equation is used:

1) to find the variation of the substrate concentration with time if K_m and r_{max} are known

or

2) to find the K_m and r_{max} constants if the substrate concentration versus time is known

If the equation is linearized:

$$\frac{1}{t}\ln\frac{C_{So}}{C_S} = -\frac{1}{K_m}\frac{C_{So}-C_S}{t} + \frac{r_{max}}{K_m}$$

Km and rmax are found

Factors Affecting Enzymatic Reaction Rate

- ✓ temperature
- ✓ pH
- ✓ liquid forces (hydrodynamic forces, surface tension,
- ✓ hydrostatic pressure)
- ✓ radiation

in addition to:

- ✓ substrate concentration
- ✓ enzyme concentration
- ✓ presence and concentrations of inhibitors and activators

Temperature Effect:

- ✓ Each enzyme has an optimum temperature at which it activates best.
- ✓ Increasing the temperature increases the enzyme activity, that is, the enzymatic reaction rate.
- ✓ However, if the temperature rises above a certain value, the heat denatures the enzyme and disrupts its three-dimensional structure by breaking the hydrogen bonds.
- ✓ Low temperature, on the other hand, reduces enzyme activity due to reduced molecular movement.

The variation of the enzymatic reaction rate with temperature is in the form of a bell curve.

In both regions, rate constant is dependent on the temperature by the Arrheinus equation.

$$k_2 = Ae^{-Ea/RT}$$

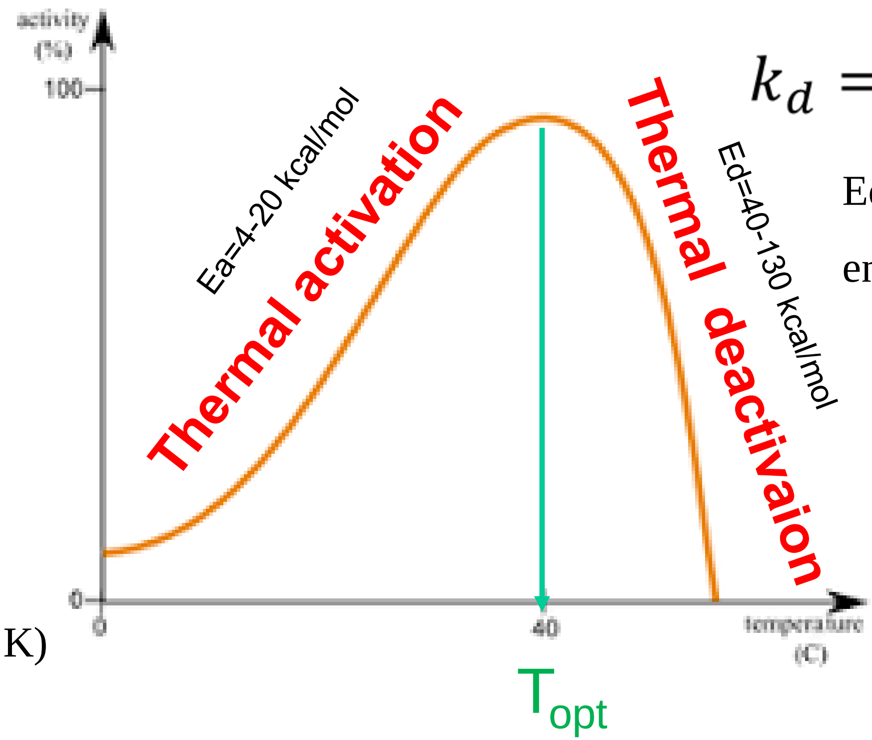
k=rate constant (k_2)

A=frequency factor

E_a= activation energy (kcal/mol)

T=absolute temperature (K)

R=ideal gas constant (1.987 cal/mol K)



$$k_d = Ae^{-Ed/RT}$$

Ed= deactivation energy (kcal/mol)