# MODULATION OF ENZYME ACTIVITY

Effectors

Inhibitor: decreases reaction rate

$v_i$

Degree of inhibition:

$$\varepsilon_i = \frac{v_0 - v_i}{v_0}$$

Activator: increases reaction rate

$v_a$

Degree of activation:

$$\varepsilon_a = \frac{v_a - v_0}{v_0}$$

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# INHIBITION

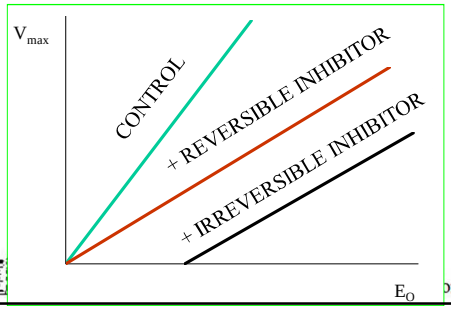
REVERSIBLE

$$\begin{array}{l} E + S \rightleftharpoons ES \longrightarrow E + P \\ \downarrow\uparrow \\ EI \end{array}$$

IRREVERSIBILE

$$\begin{array}{l} E + S \underset{}{\overset{K_S}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \\ \downarrow \\ EI \end{array}$$

distinction:




omány Tanszék

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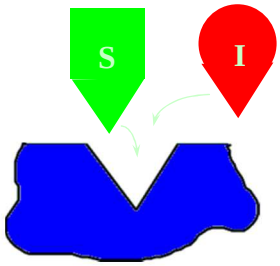
# Competitive inhibition

Competition between S and I for the active sites of the enzyme, or mutual exclusion




I may be an:

- substrate analogue
- alternative substrate
- product

MODEL 1.: Classical competitive inhibition:

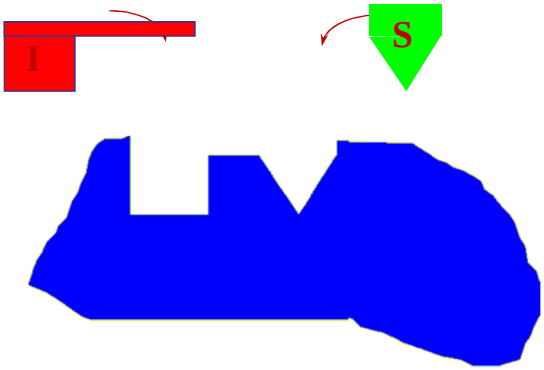
**I** competes with **S** for occupation of the same active site





# COMPETITIVE INHIBITION

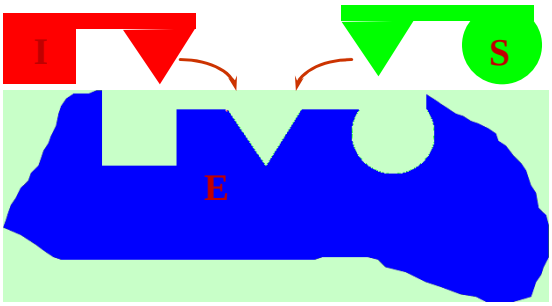
MODEL 2.: steric hindrance A




Binding of I to another site sterically hinders S in binding to the active site of enzyme.

# COMPETITIVE INHIBITION

MODEL 3.: steric hindrance B




An analog part of S and I compete for a common binding site.

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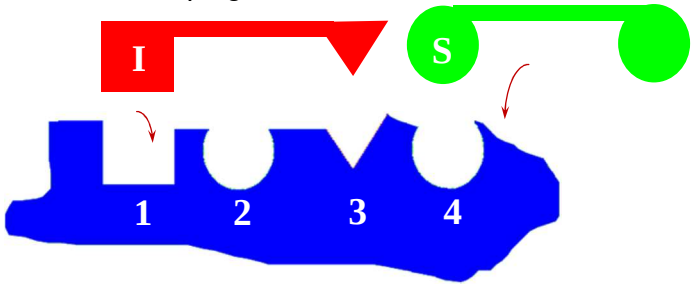
# COMPETITIVE INHIBITION

MODEL 4.: overlaping




Sites 1 and 3 can bind I, 2 and 4 can bind S, but both exclude each other.

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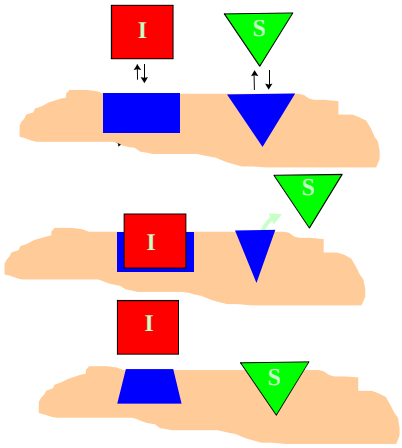
# COMPETITIVE INHIBITION

MODEL 5.:

Binding of **I** changes the conformation of the enzyme which prevents binding of **S** to the active centre.

End product inhibition (feed back inhibition) is typical example of this case.






# Kinetics of competitive inhibition

Basic equations for competitive inhibition:

$$E + S \underset{}{\overset{K_S}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$
$$+$$
$$I$$
$$\updownarrow K_i$$
$$EI \xrightarrow{k_{app}} E + P'$$

$$K_s = \frac{E \cdot S}{(ES)}$$

$$K_i = \frac{E \cdot I}{(EI)}$$

- if $k_{app}$ >0 than **I** is an alternative substrate
- if $k_{app}$ =0 than **I** is a „dead end" competitive inhibitor

# Kinetics of competitive inhibition

*Alternative substrate*: the enzyme is able to transform the structural analogous molecule, too. → an *alternative product* is formed.

$$E + S' \rightleftarrows E + P'$$

Enzymes with group and region specifity have numerous alternative substrates

Example: the enzymes of liver: alcohol dehydrogenase, aldehyde dehydrogenase: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# Kinetics of competitive inhibition

Repeat the deduction:

$$E + S \underset{}{\overset{K_S}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$
$$+$$
$$I$$
$$\updownarrow K_i$$
$$EI \xrightarrow{k_{app}} E + P'$$

$$K_s = \frac{E \cdot S}{(ES)} \qquad K_i = \frac{E \cdot I}{(EI)}$$

product formation rate:

$$V = \frac{dP}{dt} = k_2(ES)$$

Mass balance of enzyme:

$$E_0 = E + (ES) + (EI)$$

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# Kinetics of competitive inhibition

Divide the two equation:

$$\frac{V}{E_o} = \frac{k_2(ES)}{E + (ES) + (EI)}$$

Substitute:

$$K_s = \frac{E \cdot S}{(ES)} \qquad K_i = \frac{E \cdot I}{(EI)}$$

$$\frac{V}{E_o} = \frac{k_2 E \frac{S}{K_s}}{E + E\frac{S}{K_s} + E\frac{I}{K_I}} \rightarrow \frac{V}{k_2 E_o} = \frac{\frac{S}{K_s}}{1 + \frac{S}{K_s} + \frac{I}{K_I}}$$

$$V_{max} = k_2 E_o$$

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# Kinetics of competitive inhibition

Simplified forms of reaction rate:

$$\frac{V}{V_{max}} = \frac{S}{K_s\left(1 + \frac{I}{K_i}\right) + S}$$

or:

$$V = V_{max}\frac{S}{K_s\left(1 + \frac{I}{K_i}\right) + S}$$

or:

$$v_i = \frac{v_{max}(S)}{K_S\left[\frac{K_I + (I)}{K_I}\right] + (S)}$$

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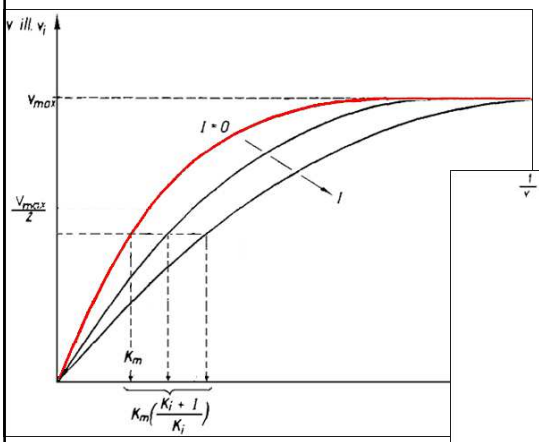
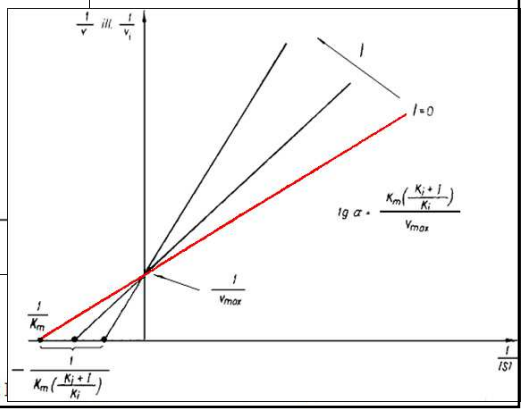
# Kinetics of competitive inhibition




$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_S}{V_{max}}\left(1 + \frac{I}{K_I}\right)\frac{1}{S}$$

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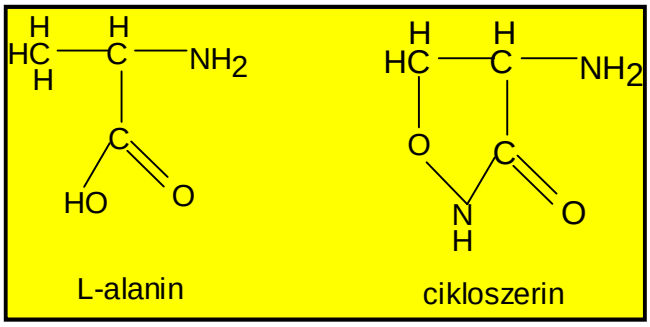
# Competitive inhibition

Alternative substrates: for hexokinase**:** glucose, fructose

S-analogons: drugs:

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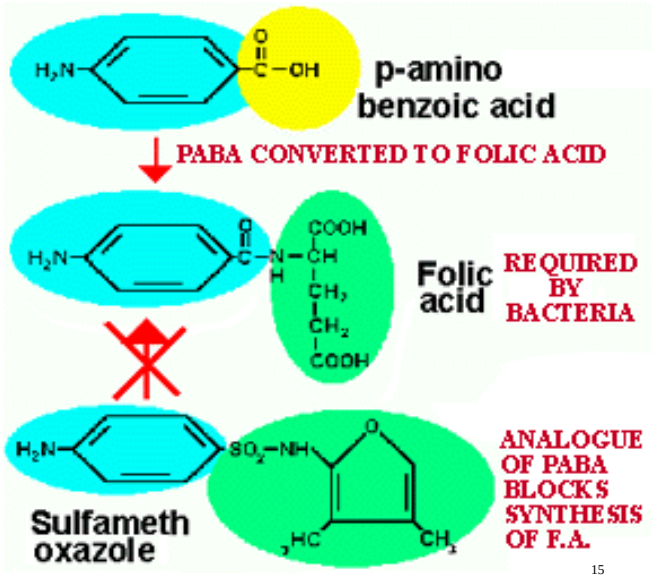
## Competitive inhibition

Effect of sulfamides (antimicrobial drugs): substrate analogon act as competitive inhibitor.

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## Analogous inhibitions

competitive inhibition:

$$V = V_{max}\frac{S}{K_s\left(1+\frac{I}{K_i}\right)+S}$$

product inhibition:

$$V = V_{max}\frac{S}{K_s\left(1+\frac{P}{K_P}\right)+S}$$

alternative or competing substrates

$$V_1 = V_{1max}\frac{S_1}{K_{S1}\left(1+\frac{S_2}{K_{S2}}\right)+S_1}$$

$$V_2 = V_{2max}\frac{S_2}{K_{S2}\left(1+\frac{S_1}{K_{S1}}\right)+S_2}$$

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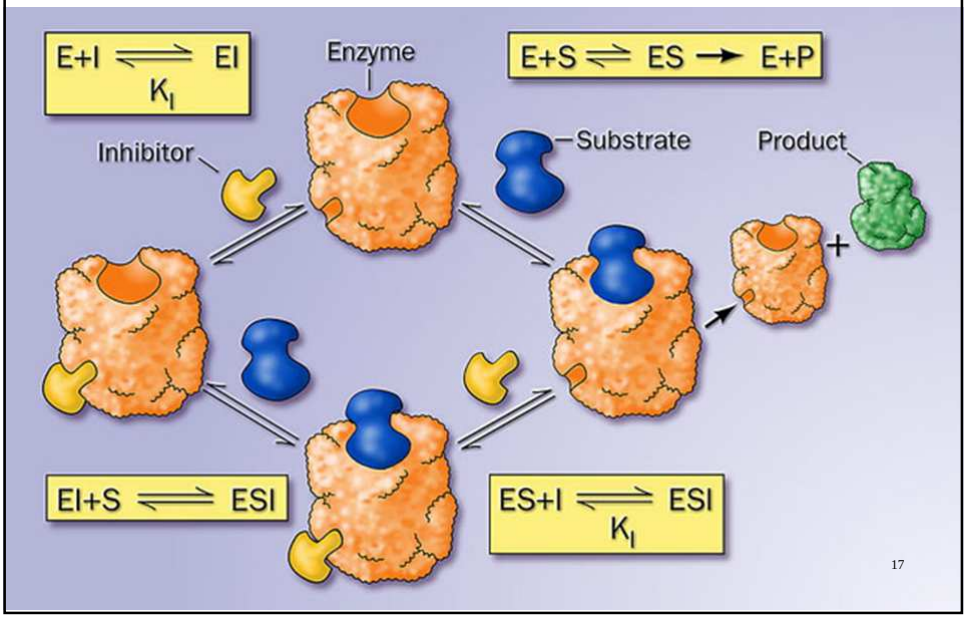
# Noncompetitive inhibition

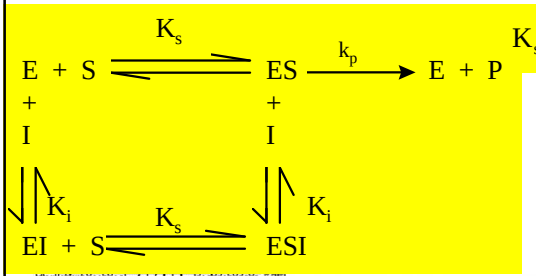



# Noncompetitive inhibition

Inhibitor binds to an other active site of the enzyme and does not affect the binding of the substrate – does not change the affinity of the enzyme to the substrate.

It exists only when rapid equilibrium can be supposed, $K_s=K_m$.

Equations of noncompetitive inhibition:




$$K_s = \frac{E.S}{ES} = \frac{EI.S}{ESI} \quad \text{és} \quad K_i = \frac{E.I}{EI} = \frac{ES.I}{ESI}$$

$$V = kp(ES)$$

$$\frac{V}{V_{max}} = \frac{ES}{E + ES + EI + ESI}$$

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## Noncompetitive inhibition

$$\frac{V}{V_{max}} = \frac{ES}{E + ES + EI + ESI}$$

$$\frac{V}{V_{max}} = \frac{\frac{S}{K_s}}{1 + \frac{S}{K_s} + \frac{I}{K_i} + \frac{S.I}{K_s K_i}}$$

Inhibitor changes the value of the apparent $V_{max}$ , but does not change the values of $K_s$ ( or $K_m$).

or

$$\frac{V}{V_{max}} = \frac{S}{K_s\left(1 + \frac{I}{K_i}\right) + S\left(1 + \frac{I}{K_i}\right)}$$

$$V = V_{maxi}\frac{S}{K_s + S} \quad \text{where} \quad V_{maxi} = V_{max}\frac{1}{1 + \frac{I}{K_i}}$$

or

$$V = V_{max}\frac{1}{\left(1 + \frac{I}{K_i}\right)}\frac{S}{K_s + S}$$

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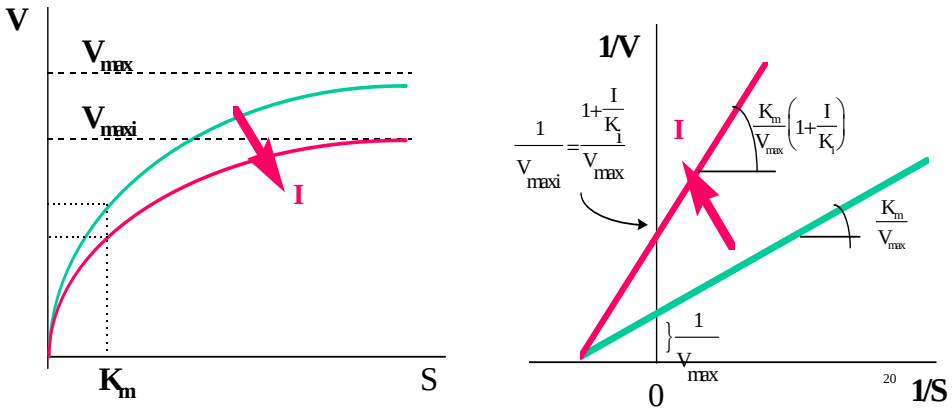
## Noncompetitive inhibition

The inhibitor affects the apparent $V_{max}$ value but does not change $K_s$ (or $K_m$).



# Noncompetitive inhibition

Examples:

**$H^+$ ions' effect on chymotripsine**. Here a proton acceptor site exists in the active centre, which can be inhibited by increasing $H^+$-ion concentration. (L-B plot shows clear noncompetitive inhibition, (but do not forget the complex effect of the pH on enzymes).

**Heavy metal molecules**(-SH reagensek), or **cyanides.**

Often these effects are irreversible.

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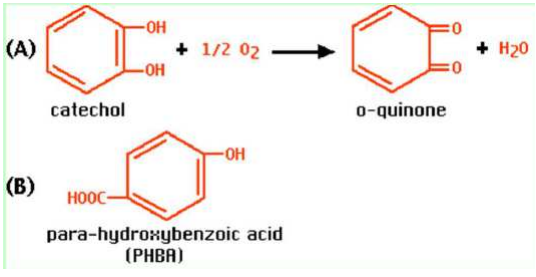
# Noncompetitive inhibition

Surface of slices apple gets brown in air: o-diphenol oxidase enzyme catalyses the catechol → o-quinone reaction




this and other reaction products give the brown color

competitive inhibitor of o-diphenol oxidase is para-hydroxy-benzoic acid (PHBA), a structural analog.

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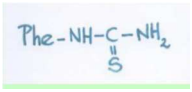
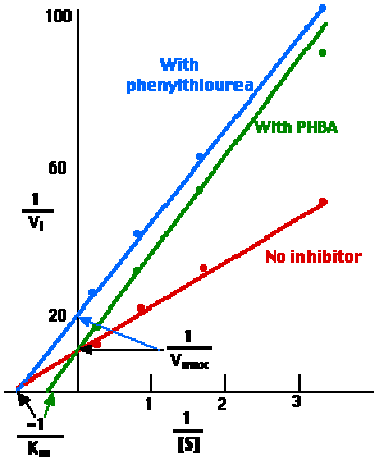
# Noncompetitive inhibition

competitive inhibitor of o-diphenol oxidase is para-hydroxybenzoic acid (PHBA), a structural analogon

noncompetitive inhibitor is: phenyl-thiourea, bound to copper ion what is necessary to enzyme activity.

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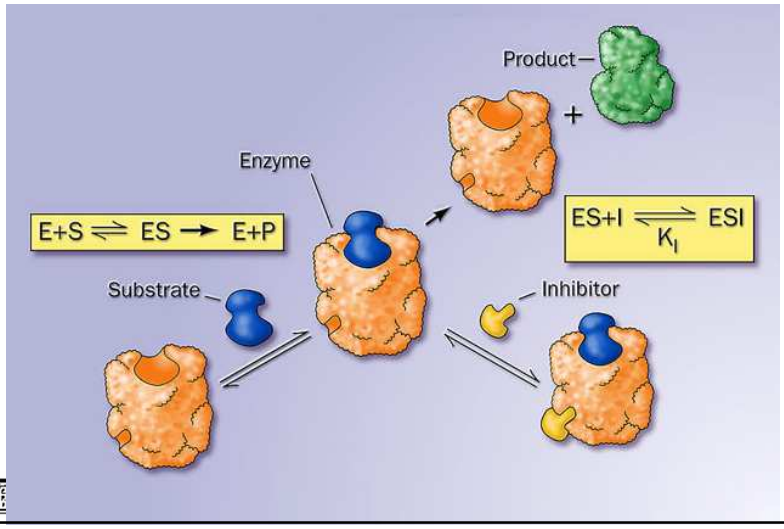
# Uncompetitive inhibition

Fixed order: the inhibitor must join second, after the substrate

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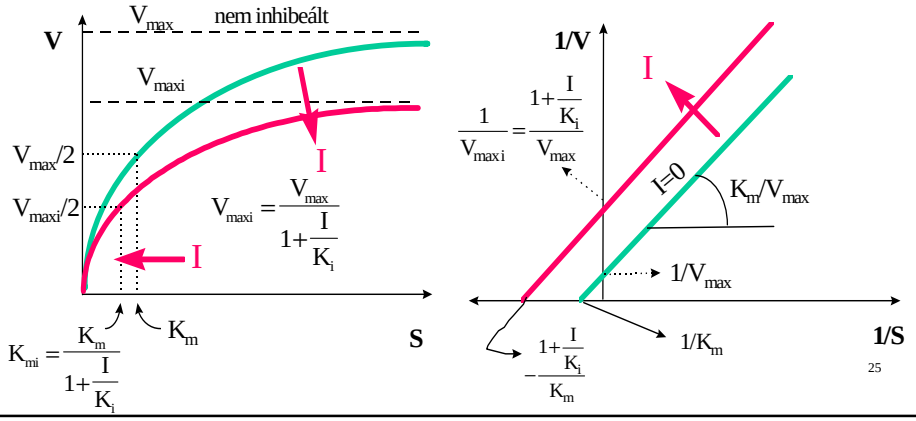
# Uncompetitive inhibition

$$V = V_{max}\frac{1}{1+\frac{I}{K_i}}\cdot\frac{S}{\frac{K_m}{\left(1+\frac{I}{K_i}\right)}+S} \qquad \frac{1}{V}=\frac{K_m}{V_{max}}\frac{1}{S}+\frac{1}{V_{max}}\left(1+\frac{I}{K_i}\right)$$

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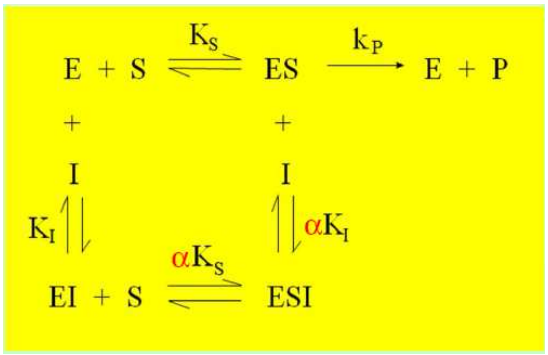
# Linear mixed type inhibition

Mechanism of linear mixed type inhibition resembles to non-competitive inhibition but presence of I modifies the enzyme affinity to substrate.

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# Linear mixed type inhibition

Expressing the change of two kinetic parameters:

$$V=V_{max}\frac{1}{\left(1+\frac{I}{\alpha K_I}\right)}\cdot\frac{S}{K_s\cdot\frac{\left(1+\frac{I}{K_I}\right)}{\left(1+\frac{I}{\alpha K_I}\right)}+S}$$

$$V_{maxi}=V_{max}\frac{1}{\left(1+\frac{I}{\alpha K_I}\right)}$$

$$K_{si}=K_s\cdot\frac{\left(1+\frac{I}{K_I}\right)}{\left(1+\frac{I}{\alpha K_I}\right)}$$

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competitive

$$V=V_{max}\frac{S}{K_s\left(1+\frac{I}{K_i}\right)+S}$$

noncompetitive

$$V=V_{max}\frac{1}{\left(1+\frac{I}{K_i}\right)}\cdot\frac{S}{K_s+S}$$

uncompetitive

$$V=V_{max}\frac{S}{K_s+S\left(1+\frac{I}{K_i}\right)}$$

$$V=V_{max}\frac{1}{1+\frac{I}{K_i}}\cdot\frac{S}{\frac{K_m}{\left(1+\frac{I}{K_i}\right)}+S}$$

mixed

$$V=V_{max}\frac{S}{K_s\left(1+\frac{I}{K_i}\right)+S\left(1+\frac{I}{\alpha K_i}\right)}$$

$$V=V_{max}\frac{1}{\left(1+\frac{I}{\alpha K_I}\right)}\cdot\frac{S}{K_s\cdot\frac{\left(1+\frac{I}{K_I}\right)}{\left(1+\frac{I}{\alpha K_I}\right)}+S}$$

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# Summary of the inhibition types

S and I mutually exclude each other from the enzyme
**COMPETITIVE**

S and I bind to the enzyme independently on each other
**NONCOMPETITIVE**

I binds only after S **UNCOMPETITIVE**

Like former but I modifies the affinity of the enzyme
**MIXED TYPE**

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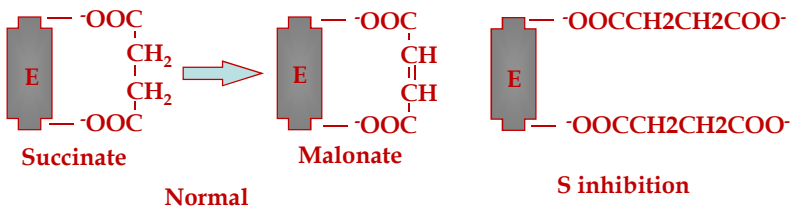
# Substrate inhibition

The substrate binds to two or more sites.
If the S concentration is high, it can occur that two S bind to one and the other binding site forming inactive complex.

(also reversible inhibition).

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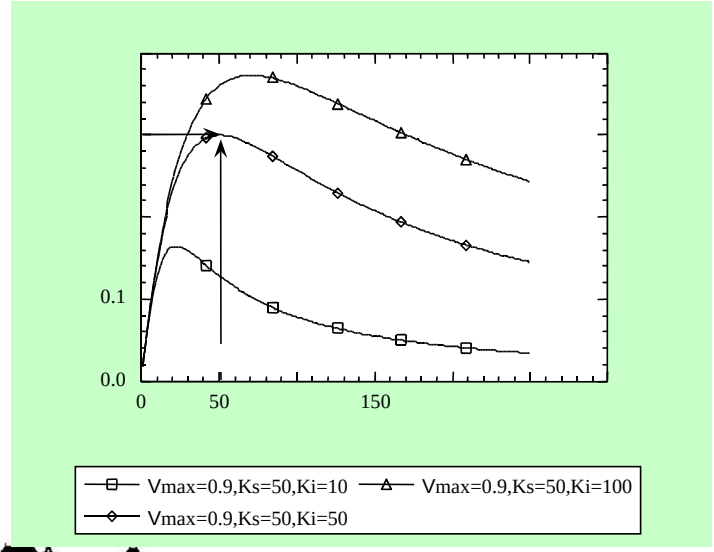
# Substrate inhibition

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