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

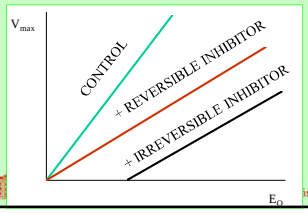
REVERSIBLE

$$E + S \rightleftharpoons ES \longrightarrow E + P$$
$$\downarrow\uparrow$$
$$EI$$

IRREVERSIBILE

$$E + S \underset{K_S}{\rightleftharpoons} ES \xrightarrow{k_2} E + P$$
$$\downarrow$$
$$EI$$

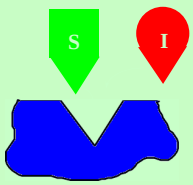
distinction:



ny Tanszék 2

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

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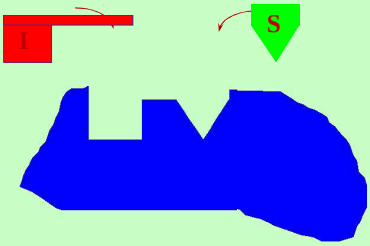
COMPETITIVE INHIBITION

MODEL 2.: steric hindrance A



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

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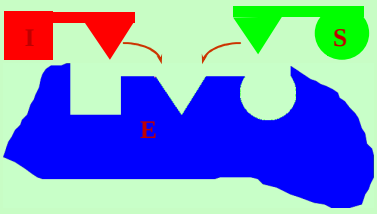
COMPETITIVE INHIBITION

MODEL 3.: steric hindrance B



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

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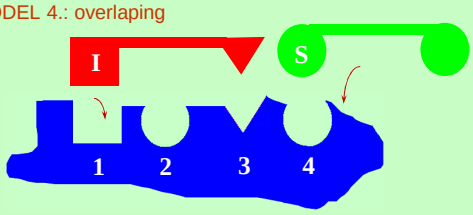
COMPETITIVE INHIBITION

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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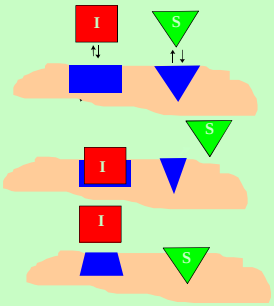
COMPETITIVE INHIBITION

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.



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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

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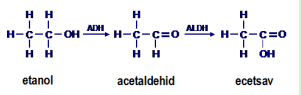
Kinetics of competitive inhibition

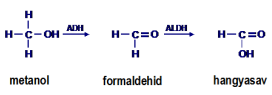
Alternative substrate: the enzyme is able to transform the structural analogous molecule, too. → an *alternative product* is formed.

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

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:

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

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

product formation rate:

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

Mass balance of enzyme: $E_0 = E + (ES) + (EI)$

Kinetics of competitive inhibition

Divide the two equation:

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

Substitute:

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

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

$V_{max} = k_2 E_o$

Kinetics of competitive inhibition

Simplified forms of reaction rate:

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

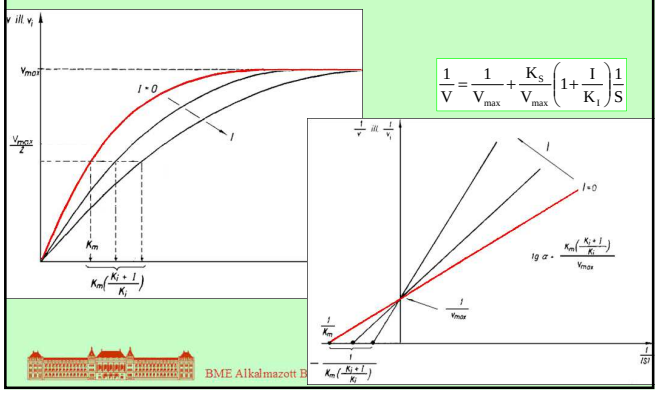
or:

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

or:

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

Kinetics of competitive inhibition

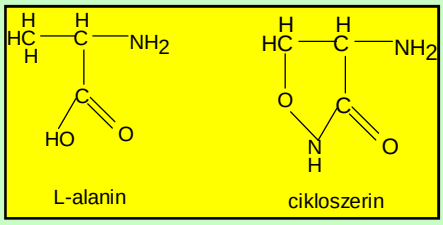


$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_S}{V_{max}}\left(1 + \frac{I}{K_I}\right)\frac{1}{S}$$

Competitive inhibition

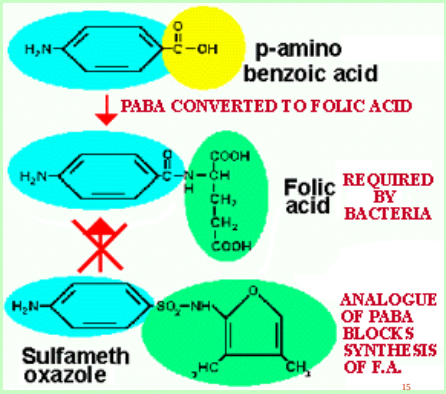
Alternative substrates: for hexokinase: glucose, fructose

S-analogons: drugs:



Competitive inhibition

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



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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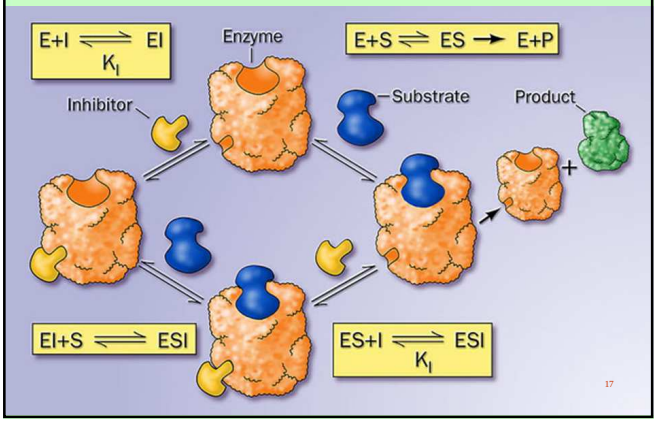
Noncompetitive inhibition



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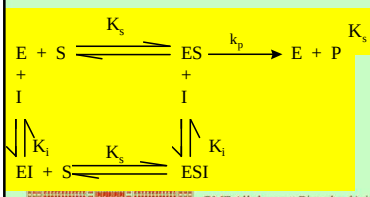
Noncompetitive inhibition

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

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

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

or

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

or

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

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

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

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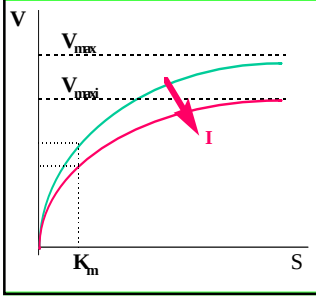
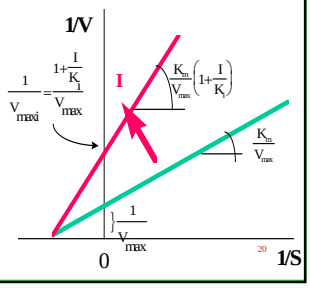
Noncompetitive inhibition

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





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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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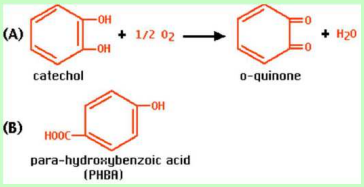
Noncompetitive inhibition

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



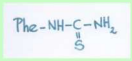
this and other reaction products give the brown color

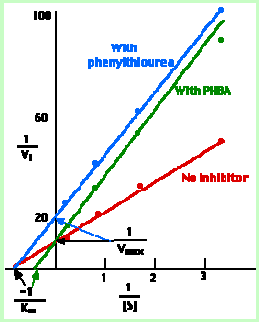
competitive inhibitor of o-diphenol oxidase is para-hydroxy-benzoic acid (PHBA), a structural analog.

Noncompetitive inhibition

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

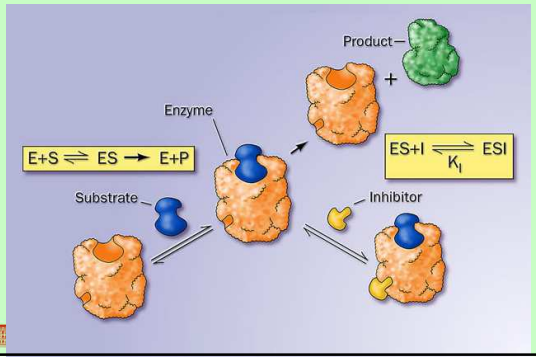
noncompetitive inhibitor is: phenyl-thiourea, bound to copper ion what is necessary to enzyme activity.





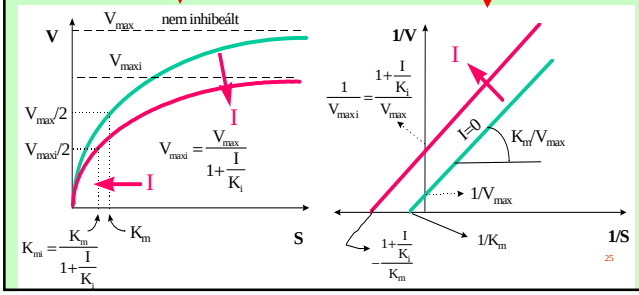
Uncompetitive inhibition

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



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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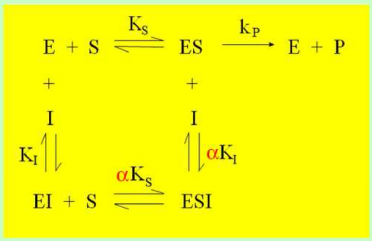
Linear mixed type inhibition

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:

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

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

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competitive noncompetitive uncompetitive

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

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

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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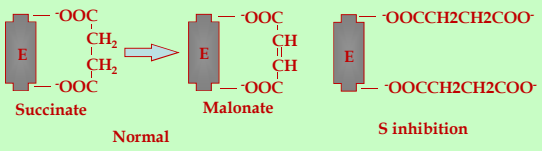
Substrate inhibition

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 comp-lex.

(also reversible inhibition).



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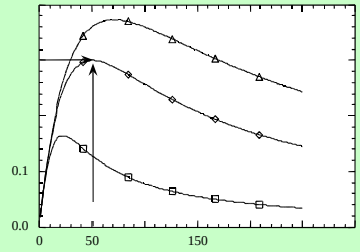
Substrate inhibition



Vmax=0.9,Ks=50,Ki=10 Vmax=0.9,Ks=50,Ki=100
Vmax=0.9,Ks=50,Ki=50

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