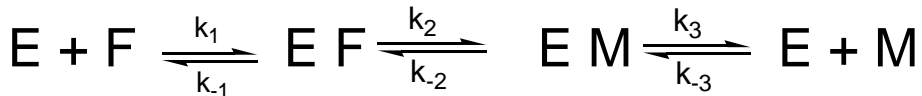
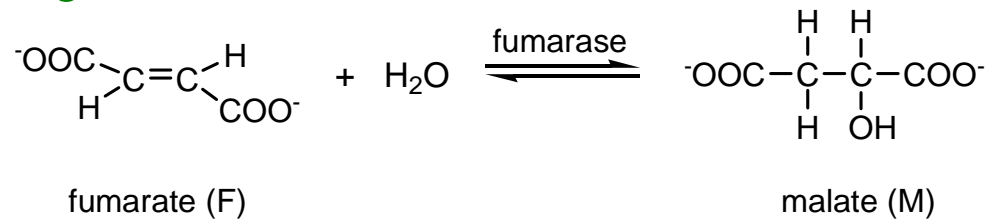


## Complex Reactions – update 2009

e.g.



Analysis gives initial rate (messy to work out, mixes fast equilibrium and steady state, forward reaction only):

$$v_F = \frac{[k_2 k_3 / (k_2 + k_{-2} + k_3)] [E_0] [F]}{\left[ \frac{k_{-1} k_{-2} + k_{-1} k_3 + k_2 k_3}{k_1 (k_2 + k_{-2} + k_3)} + [F] \right]} = \frac{V_F [F]}{K_M^F + [F]}$$

result appears simple reaction, same as Michaelis Menton form:  $v_{\text{MAX}}[S]/(K_m+[S])$  but constants complex

this experiment (init. rate) not separate intermediate

**POINT:** Relaxation measurement senses reverse reaction – can help (just do backwards, let cap. "V" be max. rate)

$$v_R = V_R [M] / (K_M^R + [M]) \quad \text{from} \quad v_R = d[F]/dt = k_{-1} [EF]$$

net rate **Remember?!**:

$$v = (V_F K_M^R [F] - V_R K_M^R [M]) / (K_M^F K_M^R + K_M^R [F] + K_M^R [M])$$

at beginning  $M = 0$  -- gives back original form

at equilibrium:  $V_F K_M^R [F]_e = V_R K_M^F [M]_e$   
 $K_e = [M]_e / [F]_e = V_F K_M^R / V_R K_M^F$

To parse out all contributions  $k_1, k_2 \dots$  etc. is difficult  
 since observations combine them

Need it isolate/detect intermediates or vary conditions  
 to get individual rate constants (ex. H/D exchange)

## Inhibition

Sometimes path involves **multiple intermediates**  
 but still apparently simple (like before)



$$v_0 = k_{\text{cat}} [E_0] [S] / (K_M + [S])$$

Difference is  $k_{\text{cat}}$  and  $K_M$  combine all  $k_i$  above  
 if one step (i) is slow (rate limit)  $k_{\text{cat}} = k_i$   
 $\rightarrow$  turnover depends on slow step

$K_M$  always like (apparent) dissociation constant  
 for all enzyme bound species:  $[E][S]/[ES]$

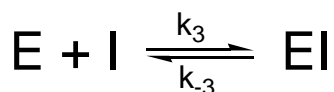
if  $k_{-1} \gg k_i$  then  $K_M = k_{-1}/k_1$  true dissociation const.

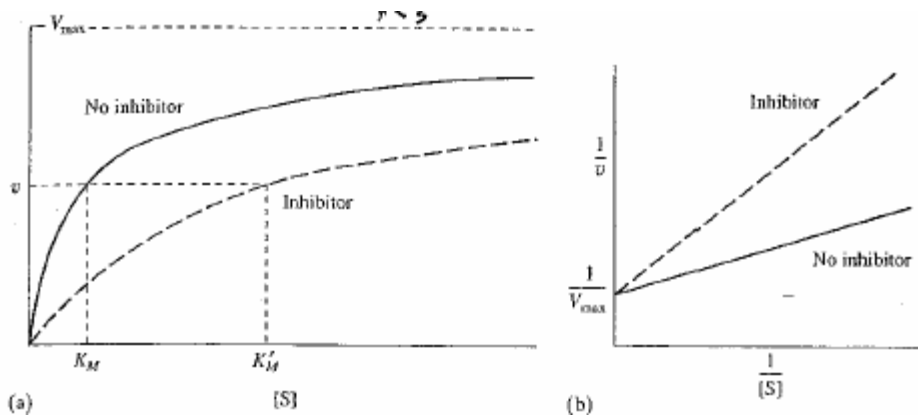
Now many enzymes **bind several different substrates**  
 but only one gives product  $\rightarrow$

Some substrates called **inhibitor bind tightly**

but have **no reaction**  $\rightarrow$  remove  $E_0$  - not avail. for S

**Competitive inhibition, parallel mechanism**





### No Inhibitor and Inhibitor comparison

- plot rate  $v_0$  vs.  $[S]$  and  $1/v_0$  vs.  $1/[S]$  see comparison.
- Inhibitor raises slope of Lineweaver Burk plot but same intercept – change  $K_m$  not  $v_m$

Note half velocity,  $v_0 = v_m/2$ , intercept on  $v_0$  vs.  $[S]$  plot =  $K_m$

do steady state on  $[ES]$  again

$$v = k_2[ES] \quad [ES] = k_1[E][S]/(k_{-1} + k_2)$$

but now  $[E_0] = [E] + [ES] + [EI]$  reduce effect. enzyme (EI takes some of  $E_0$  preventing its forming ES)

let  $K_I = [E][I]/[EI]$        $K_M = [E][S]/[ES]$  -- 2 dissoc. const.

$$[E_0] = [E](1 + [I]/K_I) + [ES]$$

--  $[E]$  avail.  $[ES]$  reduced--consider  $E \xrightleftharpoons[k_{-1}]{k_1} ES$  equil.

$$[E] = [E_0] - [ES]/(1 + [I]/K_I)$$

substitute in:  $[ES] = K_M^{-1}([E_0] - [ES])[S]/(1 + [I]/K_I)$

let denom.:  $\alpha = (1 + [I]/K_I)$  --  $[ES] = [E_0][S]/(1 + [S]/\alpha K_M)$

Note  $\alpha > 1$ , inc.  $[I]$  inc.  $\alpha$ , decrease  $v_0$

Then looks like before:  $v_0 = k_2[E_0][S]/(\alpha K_M + [S])$

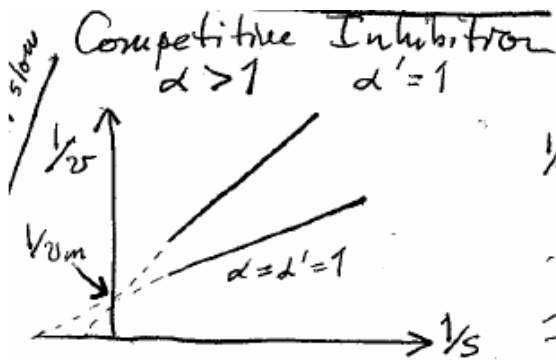
but  $\alpha K_M = K'_M$  will mean  $1/S$  vs.  $1/v$  has diff. slope

fits observation – half rate  $\Rightarrow K'_M$

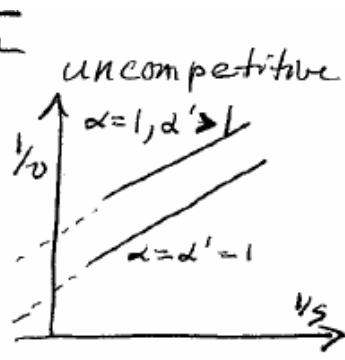
As  $[I]$  increases  $I \ll K_i \Rightarrow \alpha \sim 1 \rightarrow$  ordinary M - M  
 $I \gg K_i \Rightarrow \alpha \sim [EI]/[E] \gg 1$   
 so  $[EI] \gg [E]$

Fig. 8.20 Atkins add:  $ES + I \rightleftharpoons ESI$

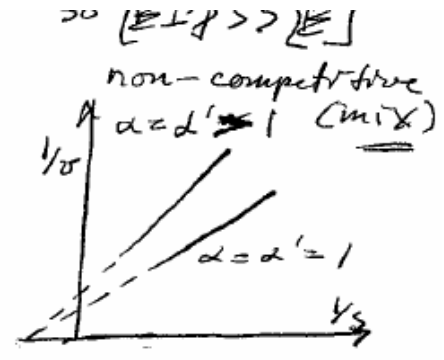
Competitive Inhibition    uncompetitive    non-competitive



as described above



here no EI form ( $\alpha = 1$ )  
 but ESI form ( $\alpha' > 1$ )



here both EI & ESI form

as described above

here no EI form ( $\alpha = 1$ )  
 but ESI form ( $\alpha' > 1$ )

here both EI  
 and ESI form

Inhibition hugely important –

most biochemistry processes need enzymes →

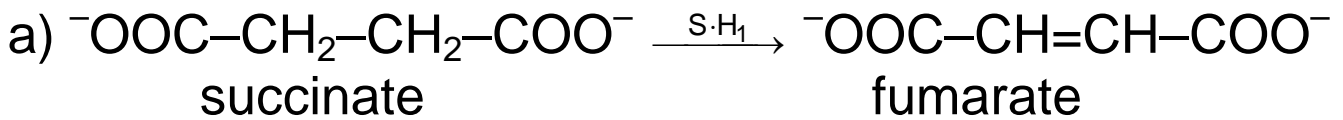
- need specific reaction to go under control
- need operate with low concentration enzyme
- need fast – S with response on/off

control – bio feedback/signaling

– gone wrong – “sick”

Drugs/pharma intercede → inhibit

e.g. succinic dehydrogenase

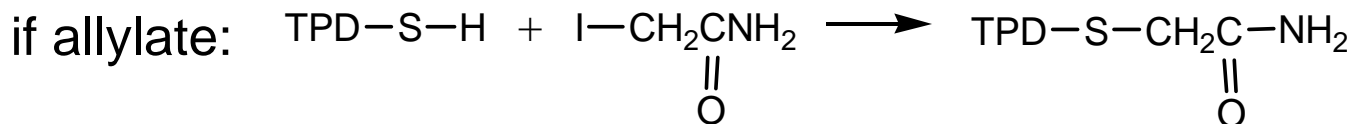


if add malonate:  $\text{OOC-CH}_2\text{-COO}^-$

has similar shape – binds active site  
but cannot dehydrogenate (no C=C)

so stays on enzyme: EI / inhibit form succinate: ES  
like case (a)

b) Triosephosphate dehydrogenase has Cys in active site

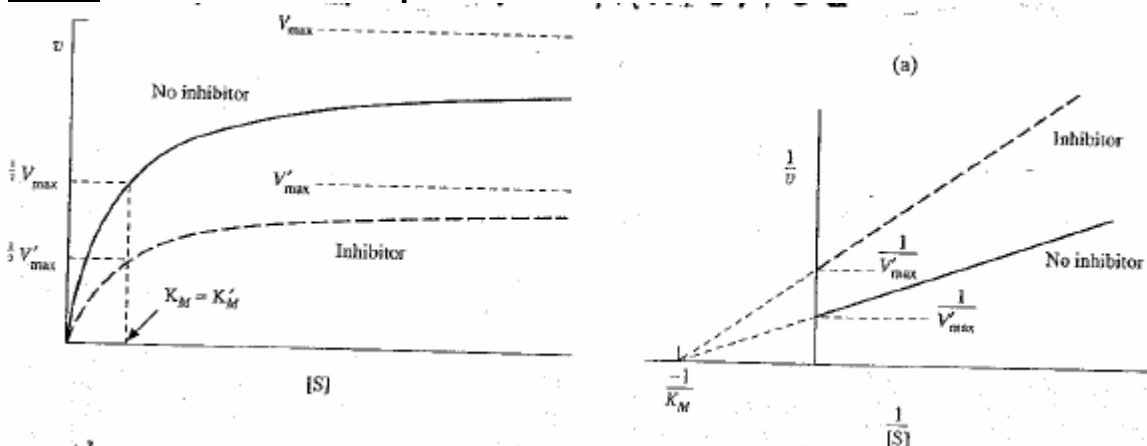


This enzyme now lost to reaction inhibition (no reversal)

but  $K_M$  same,  $v_{\max}$  reduced

$\Rightarrow$  like case (c) – non-competitive  $\alpha, \alpha' > 1$

Plot for non-competitive inhibition



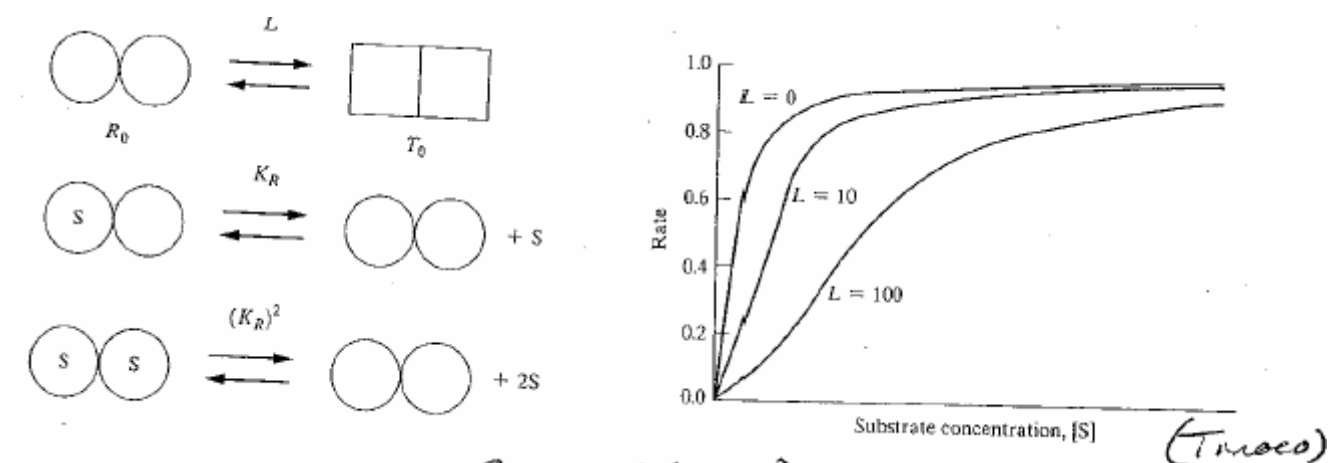
$K_M$  same,  $V_{\max}$  reduced

**Allosterism** → binding substrate to one site/subunit affect binding/release at 2nd or 3rd ...  
 e.g. hemoglobin – 4 subunits, each bind O<sub>2</sub> → cooperate

MWC model → each subunit 2 forms/different binding efficiency

R – relaxed – binds better

T – tense                      no substrate:  $L = [T_0]/[R_0]$  equilibrium



Solve mechanism use pre-equilibrium:

$$v = v_{\max} \alpha (1 + \alpha) / \{ (1 + \alpha)^2 + L \} \quad - \alpha = [S]/K_R$$

$L = 0 \rightarrow$  no T  $\rightarrow v = v_m \alpha / (1 + \alpha)$     this is M-M

L increase  $\rightarrow$  sigmoidal shape in rate vs. [S]

$\rightarrow$  binding S convert T  $\rightarrow$  R  $\Rightarrow$  max rate constant

Many topics in biochemistry have time/rate dependence

- a) diffusion → rate material moves in medium
  - faster motion more “friction” affect equilibrium
  - ⇒ separations like – electrophoresis
    - HPLC
  - ⇒ pharmacological – cross blood-brain barrier
    - drug absorption
  
- b) charge transfer – many reactions are re-dox move charge in enzyme -- special method- Marcus theory
  - also move charge in cell through  $\text{Na}^+$  or  $\text{K}^+$  channel

Summary: Rate laws – observed behavior  
T-dependence – Arrhenius  
Absolute Rate Theory – interpret  
Mechanism – put steps together  
Enzymes – one application