Bio 5312 Enzyme Kinetics

Two substrate reactions

Dihydrofolate reductase- a two substrate example

Data analysis - full time course

Allosteric enzymes – one and two substrates

Phosphofructokinase as an allosteric enzyme

Bio 5312 Enzyme Kinetics

Texts: For single substrate enzymes and basic concepts, any Biochemistry textbook will do.

For multisubstrate enzymes as well as issues related to mechanisms:
Fersht (1999) "Enzyme Structure and Mechanism"
Leskovac (2003) "Comprehensive Enzyme Kinetics"
Cornish-Bowden (2004) "Fundamentals of Enzyme Kinetics"
Cook and Cleland (2007)"Enzyme Kinetics and Mechanism"

For transient kinetics: Johnson review (handout).

For a review of enzyme kinetics: Johnson "A century of enzyme kinetic analysis, 1913-2013"

To access papers for class, go to Bio 5312-Macromolecular Interactions. Click on journal articles and images.

You have already had some information to prepare for enzyme kinetics. Two substrate cases are more complicated than single substrate cases.

The vast majority of enzymes involve 2 or more substrates – even if the second substrate is H_2O.

Dr. Galletto described E + S == ES
And determination of rate constants for binding steps and a conformational change. Can measure by, for example, fluorescence change on ligand binding

Lohman discussed diffusion controlled steps with a rate constant of $10^6 - 10^8$ /sec-mol

What about other steps in an enzymatic reaction
What is rate limiting
E + S == ES is usually a fast step, but is second order and therefore depends on concentration

ES==EP You might expect that the chemical transformation would be a rate determining step. But frequently not

EP == E + P Leaves the off rate as rate limiting.

We have ignored what might be the most important rate determining steps – conformational changes.

So, as Dr. Galletto described

E+ S ==ES == ES'

Which gives two apparent rate constants each of which includes all the rate constants for the process. If you can determine the two apparent rate constants as a function of the substrate concentration, you can solve for all 4 intrinsic rate constants.

For a multisubstrate enzyme you should be able to do this experiment for each substrate and for the products as well.

Two substrates - I

Some reminders

Single substrate case

The Michaelis-Menten equation

$V_0 = V_{max}/(1+K_m/S)$

The Michaelis constant has the units of concentration
It is simply defined as the concentration at which the initial velocity is one-half the maximum velocity.

A simple rapid equilibrium derivation is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

$v_0 = k_2(ES)$ (no reverse step)

K_S = a dissociation constant $= \frac{(E)_f(S)_f}{(ES)}$

$v_0 = k_2 \frac{(E)_f(S)_f}{K_S}$

$E_0 = E_f + ES$ (ignore EP)

$= E_f\left(1 + \frac{S}{K_S}\right)$. $E_f = \frac{E_0}{1 + \frac{S}{K_S}}$

$v_0 = k_2 \frac{(E)_0 S}{K_S}\left\{\frac{1}{1 + \frac{S}{K_S}}\right\}$

$= \frac{k_2(E_0)}{1 + \frac{K_S}{S}}$

Kinetic data can not "prove" a mechanism

Single substrate
Rapid Equilibrium
For the mechanism $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$

$V_m = k_2(E_0)$
$K_m = k_{-1}/k_1 = K_d$

Steady State
For the mechanism $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$

$V_m = k_2k_3(E_0)/(k_2+k_{-2}+k_3)$
$K_m = k_{-1}k_3 + k_{-1}k_{-2} + k_2k_3/k_1(k_2 + k_{-2} + k_3)$

The complete steady state derivation is given Bio5312 – other

Two or more substrates

Types of mechanisms:

Ordered $E + A \rightleftharpoons EA$

$EA + B \rightleftharpoons EAB \ldots$

Random $E + A \rightleftharpoons EA$

$E + B \rightleftharpoons EB$

$EA + B \rightleftharpoons EAB$

$EB + A \rightleftharpoons EAB \}\ldots$

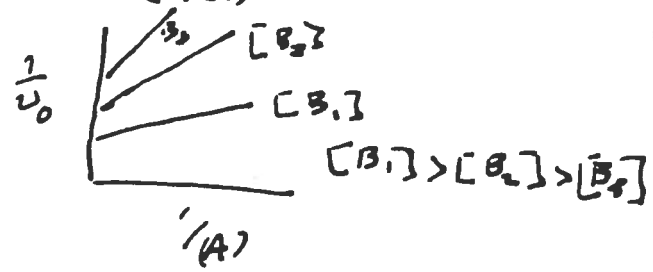
Ping pong $E + A \rightleftharpoons EA \rightleftharpoons E' + P$

$E' + B \rightleftharpoons E'B \rightleftharpoons E + Q$

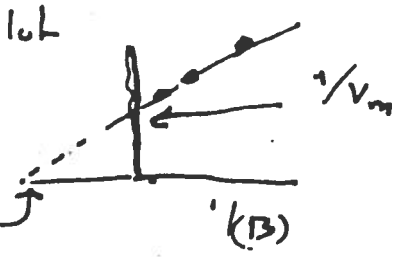
A "typical" equation

$$v_0 = \frac{V_m}{1 + \frac{K_A}{(A)} + \frac{K_B}{(B)} + \frac{K_{AB}}{(A)(B)}}$$

Primary plot



Secondary plot



But The initial velocity expression depends on the assumption used in the derivation.

The equation for v_0 can be derived for

1) Rapid equilibrium random substrate addition

OR

2) Steady-state ordered addition

For Rapid Equilibrium ordered

$$v_0 = \frac{V_m}{1 + \frac{K_m}{(A)} + \frac{K_{AB}}{(A)(B)}}$$

For Steady-state random, the equation contains terms in $A, A^2, A^2B, AB, B, AB^2, B^2$

For ping-pong

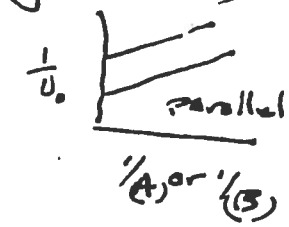
$$v_0 = \frac{V_m}{1 + \frac{K_m}{(A)} + \frac{K_B}{(B)}}$$

How to distinguish between These mechanisms
(and is That important)

Ping - pong is easy (and not very common)

Ligand binding (substrate)

Product inhibition



Bottom line:

Random: competition between
all substrates and products

Ordered: Q vs A is competitive

P vs B is non-competitive

The full rate equations (including product)

Single substrate

$$v = \frac{\frac{V_s}{K_s}(S) - \frac{V_r}{K_r}(P)}{1 + \frac{K_s}{S} + \frac{K_p}{P}} = v_f - v_r$$

Bi - bi ordered - steady state

$$\frac{V_1 V_2 (A \cdot B - P \cdot Q / K_{eq})}{K_{ia} K_b V_2 + K_b V_2 A + K_a V_2 B + V_2 A \cdot B + \frac{K_q V_1 P}{K_{eq}} + \frac{K_p V_1 Q}{K_{eq}} + \frac{V_1 P Q}{K_{eq}}}$$

$$+ \frac{K_q V_1 A \cdot P}{K_{ia} K_{eq}} + \frac{K_a V_2 B \cdot Q}{K_{iq}} + \frac{V_2 A \cdot B \cdot P}{K_{ip}} + \frac{V_1 B \cdot P \cdot Q}{K_{ib} K_{eq}}$$

Note: 1) Change in nomenclature

2) terms in (AP); (BQ), (ABP), (BPQ)

For $P = Q = 0$

$$v = \frac{V_1 V_2 A \cdot B}{K_{ia} K_b V_2 + K_b V_2 A + K_a V_2 B + V_2 A B}$$

$$= \frac{V_1}{1 + \frac{K_a}{A} + \frac{K_b}{B} + \frac{K_{ia} K_b}{A B}}$$

For $P = 0$ (after rearranging)

$$v = \frac{V_1}{1 + \frac{K_a}{A}\left(1 + \frac{Q}{K_{iq}}\right) + \frac{K_b}{B} + \frac{K_{ia} K_b}{A \cdot B}\left(1 + \frac{K_p V_1 Q}{K_{ia} K_b K_{eq} V_2}\right)}$$

and for $f(A)$ at constant B

$$v = \frac{V_1 / \left(1 + \frac{K_b}{B}\right)}{1 + \frac{K_a}{A}\left\{\frac{\left(1 + A/K_{iq} + \frac{K_{ia} K_b}{B}\left(1 + \frac{K_p V_1 Q}{K_{ia} K_b K_{eq} V_2}\right)\right)}{1 + \frac{K_b}{B}}\right.}$$

- Q is a competitive inhibitor vs A - (at B = constant)

At saturating B

$$v = \frac{V_1}{1 + \frac{K_a}{A}\left(1 + \frac{Q}{K_{iq}}\right)}$$ competitive !

For $Q = 0$

Rearranging as before

$$v = \frac{V_1}{1 + \frac{P}{K_{ip}} + \frac{K_a}{A} + \frac{K_b}{B}\left(1 + \frac{K_q V_1 P}{K_{ia} K_q}\right) + \frac{K_{ia} K_b}{A \cdot B}\left(1 + \frac{K_q V_1 P}{K_{ia} K_b K_q V_2}\right)}$$

or

$$\frac{V_1 / \left(1 + \frac{P}{K_{ip}}\right)}{1 + \frac{K_a}{A}\left(\frac{1}{1 + \frac{P}{K_{ip}}}\right) + \frac{K_b}{B}\left\{\frac{1 + \frac{K_q V_1 P}{K_{ia} K_q}}{1 + \frac{P}{K_{ip}}}\right\} + \frac{K_{ia} K_b}{A \cdot B}\left\{\frac{1 + \frac{K_q V_1 P}{K_{ia} K_b K_q V_2}}{1 + \frac{P}{K_{ip}}}\right\}}$$

mixed inhibition

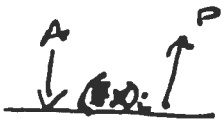
i.e., at $A = \infty$ P is not competitive vs B

why?

at $B = \infty$ P is uncompetitive vs A

Some nomenclature, short hand

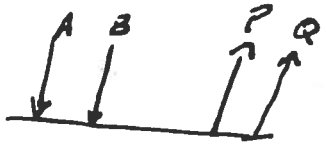
Single substrate



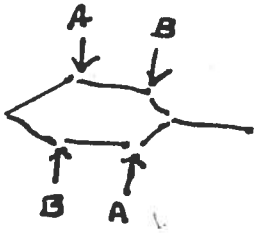
The form of the equation is always the same regardless of the number of intermediates $(EX)_i$

Two substrate

Ordered

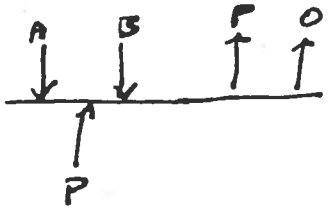


Random



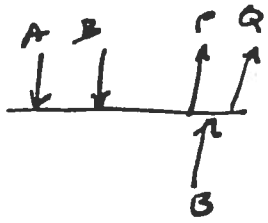
Preferred path

Some complications: two substrate, ordered



P binds to EA complex

or



B binds to EQ

EAP

EQB

dead end or abortive complexes

there is more information about mechanism in the full time course of a reaction than in the initial velocity.

It is also better to determine individual rate constants rather than kinetic parameters (the V_m and K_m).

What's important to remember

Distinguish rates from rate constants.

$A \rightarrow P$, $-d(A)/dt = k[A]$ where k is a first order rate constant with the units of t^{-1}
$A+B \rightarrow AB$, $d(AB)/dt = k[A][B]$ where k is a second order rate constant $t^{-1}M^{-1}$

For the single substrate case

1. The Michaelis constant is probably not a dissociation constant.
2. Velocity expressions are the same irrespective of the method of derivation. They are also the same irrespective of how many intermediates exist between EA and EP.

   The rapid equilibrium assumption is just that: assumes steps E+A and E+P are in rapid equilibrium (K_m is a dissociation constant)
   The steady state derivation makes no such assumptions.

For reactions involving 2 or more substrates

1. The Michaelis constant for one substrate may (or may not) depend on the concentration of the other substrate.
2. There are different mechanisms involving substrate addition or product release.
3. The initial velocity expression does depend on the method of derivation.
4. The expression for the velocity can be very complicated. Determination of the individual rate constants is much more revealing in terms of the kinetic mechanism.

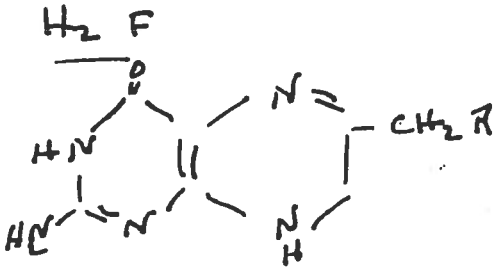
DHFR-1

The application of these methods to the study of *E. coli* dihydrofolate reductase

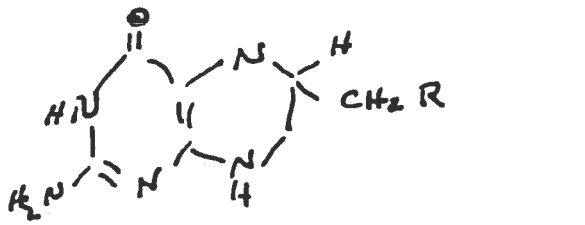
MWt = 18000

The overall reaction:

$$NADPH + H^+ + H_2F \rightleftharpoons NADP + H_4F$$



H_2F



5, 6, 7, 8 tetrahydrofolate

Reaction followed by ΔA_{340} (Absorbance) $\cong 12/mM$

Ligand binding followed by fluorescence.

Quenching of tryptophan fluorescence is biphasic with substrate

1st phase = ligand binding

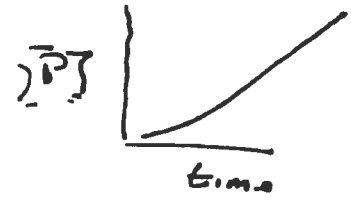
2nd phase = conformational change

Presumed mechanism

E' ⟵ slow step, $t_{1/2} \cong$ several seconds

$E + L \rightleftharpoons EL$

Furthermore, the enzymatic reaction shows a time dependent increase in activity



which means that it is difficult to say what the initial velocity is.

However, the lag can be abolished by preincubating the enzyme with either substrate

∴ all kinetic (stopped-flow) experiments involve preincubation with one of the substrates.

Since either substrate (or product) quenches the fluorescence, the kinetic mechanism must be <u>random</u>.

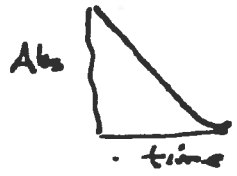
K_d's can be determined from this type of experiment, but there are conditions that must be met for tight binding ligands

i.e., the enzyme concentration should be <u>less than the</u> K_d.

K_D values:

NADPH	0.03 - .1	μM	
H_2F	0.3	μM	
NADP	2	μM	} products bind tightly
H_4F	0.2	μM	} products bind tightly

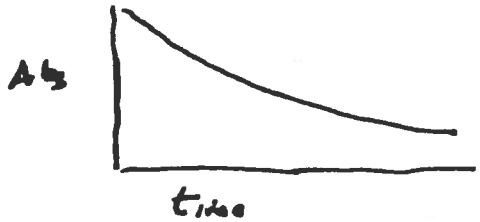
Making the assumption that these values are similar to the Michaelis-Menten K_m's and starting with 20 μM NADPH and H_2F would expect



Abs = absorbance

i.e., the velocity will only slow down when the substrate concentrations get close to the K_m values. Since these are very small, this would happen only near the very end of the full time course.

But, experimentally observe



which means either that K_m values are very high (to experiments exclude this). or that product buildup is affecting the reaction

What is the rate limiting step?

Substrate binding?

Probably not because the "on" rate constants are on the order of $5\text{-}10 \times 10^7\ s^{-1} M^{-1}$

Chemical transformation?

Use single turnover or burst experiments

$k > 200\ s^{-1}$

but $V_{max} = 15\ s^{-1}$

∴ can not be rate limiting.

Product release?

For example, $E \cdot H_4F \rightarrow E + H_4F$

$K_d = 0.2\ \mu M = \frac{k_{off}}{k_{on}}$

If $k_{on} = 10^7\ s^{-1} M^{-1}$,

$k_{off} = 10^7 \times 2 \times 10^{-7} = 2\ s^{-1}$

which is slower than V_{max}.

Can experimentally measure off rate by a displacement reaction and it is found to be $5\ s^{-1}$ still slower than V_{max}

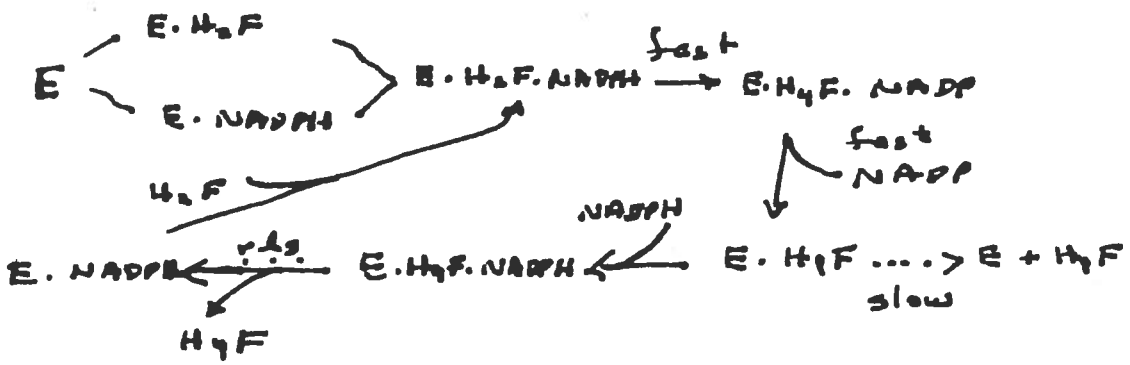
The answer is the formation of <u>abortive complexes</u>

$E \cdot NADP \cdot H_2F \longrightarrow E \cdot NADP + H_2F \quad k_{off} \simeq 50\,s^{-1}$

$E \cdot NADPH \cdot H_4F \longrightarrow E \cdot NADPH + H_4F \quad k_{off} \simeq 150\,s^{-1}$

AH - HA!
This could be rate limiting.

So the full mechanism



∴ never any free enzyme

The formation of the abortive complex is advantageous.

Single molecule experiments

What are they? Protein tethered to solid surface

Experiments with DHFR.

Methotrexate is an analog of folate and is a potent inhibitor of DHFR

The protein was engineered in the following way

1) the 2 cysteine residues were replaced with either serine or alanine

2) A cysteine was inserted at position 18

3) A 19 aa tether containing a lysine biotinylation site was attached to the N-terminal end

4) The cysteine was modified with Alexa 488

5) The protein was biotinylated and attached to a glass surface through a biotin-streptavidin-biotin sandwich

E. coli DHFR

Some effects of mutations at conserved residues

	Hydride transfer	k_{off} (H$_4$F)	V_{max}	K_D (CH$_2$F)
wt	$> 500\ s^{-1}$	$120\ s^{-1}$	$120\ s^{-1}$	0.2 μM
F31V	400	> 20	326	7
L54G	14	> 300	14	140
T113V	120	60	32	30

For L54G, the hydride transfer is the rate limiting step

See DHFR-the movie

DHFR
The mechanism and the role of protein motions

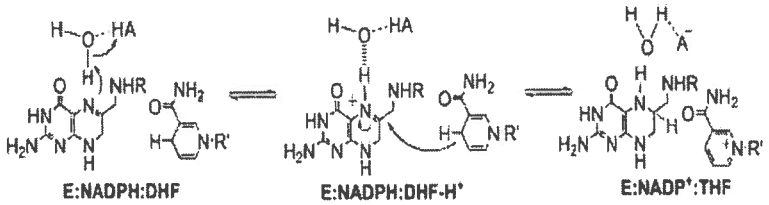
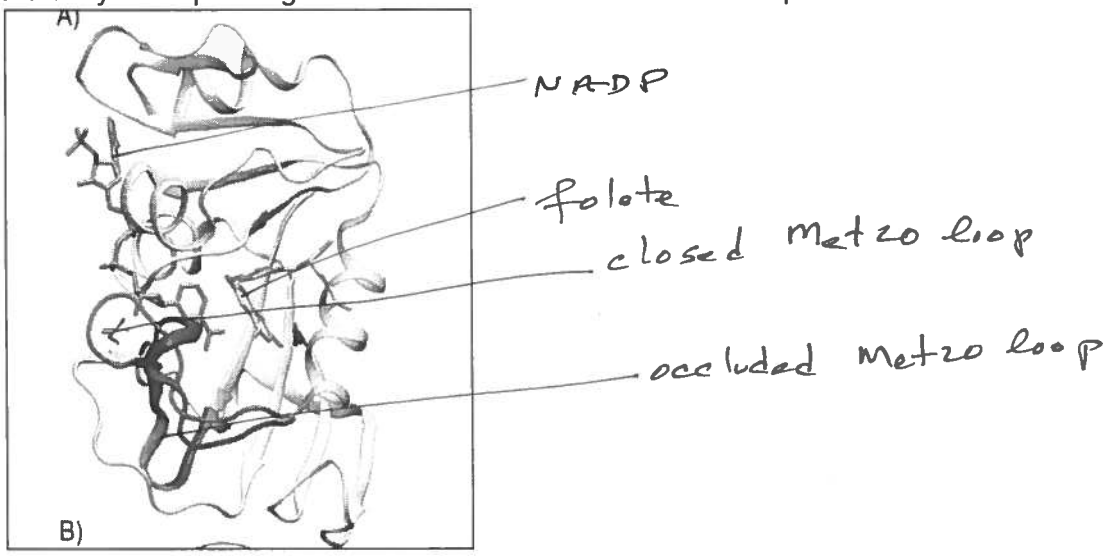


Figure 1. Catalytic mechanism of *ec*DHFR involving proton transfer from an active site water molecule to N5 of DHF (blue) and subsequent hydride transfer from NADPH to C6 of DHF (red).

From Hanoian et al. Acc Chem Res 2015 *48* 482-489

Motion and flexibility have important functional roles in substrate binding, the chemical transformation and product release. There are numerous different time scales with in a protein ranging from femtoseconds to milliseconds to seconds. The fastest motions are vibrational motions probably not related to enzymatic activity. Slower time scales are small conformational changes and slow time scales are probably domain motions. For DHFR, the pKa of the N5 atom of the substrate DHF varies from 2.6 in buffer to up to 9 in the enzyme depending on the conformation of the Met20 loop.



Some mutations affect the off rate
WT k_{off} (H_4F) 12 sec^{-1}
L54G >300 sec^{-1}

Other affect hydride transfer

Conserved residues include Met 42 and Gly121. What happens when these (and other residues are modified. Biochemistry *41* 2002 12618-12628

Table 5: Forward Hydride Transfer Rates (k_{hyd}) at 25 °C in MTEN Buffer, pH 7.0

DHFR	k_{hyd} (s^{-1})	k_{hyd}(WT/mutant)
WT	228 ± 8	1.0
M42F	159 ± 17	1.4
M42W	5.6 ± 0.4 (31)[a]	41
G121A	38 ± 3	6.0
G121S	3.7 ± 0.4	62
G121V	1.4 ± 0.2 (3.5)	163
G67V (*12*)	190 ± 15	1.2
M42W-G67V	3.9 ± 0.4 (65)	59
M42F-G121A	1.3 ± 0.2	175
M42F-G121S	0.46 ± 0.08	496
M42W-G121A	0.27 ± 0.04 (0.60)	844
M42W-G121S	0.07 ± 0.01 (0.64)	3257
M42W-G121V	0.030 ± 0.005 (0.50)	7600
G67V-G121S	2.7 ± 0.2	84

[a] Rate constants for a conformational change step preceding hydride transfer are indicated in parentheses.

See DHFR-the movie

Single turnover experiments

Done under conditions where the enzyme concentration is equal to or greater than the substrate concentration. The idea is to measure the rate of the chemical transformation.

$$E > \text{or} = A$$

For the simple case

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP$$

Under these conditions where no product is formed (all product is tied up in the EP complex)

Assuming that there is an observable change between EA and EP and, for the sake of simplicity that k_{-2} is zero.

Assume k_1 is ~ diffusion controlled = ~ 10^7 $sec^{-1}mol^{-1}$. k_{-1} is not particularly important but should be small enough that an appreciable amount of EA is formed prior to the chemical transformation.

The formation of EA would probably be too fast to measure by standard stopped flow experiments – i.e., faster than 1 msec. Thus one measures EA→EP.

In vivo it is very possible that the enzyme concentration is greater than the substrate concentration. Under these conditions the Michaelis-Menten equation does not hold.

There is no explicit equation that describes the complete enzymatic reaction. The complete description of the time course of an enzymatic reaction requires using numerical integration programs.

Numerical integration:
For any process

$$X_1 + \ldots == X_2 + \ldots$$

There is a flux for every species such that

$d(X_i)/dt$ = sum of all steps leading to formation of X_i minus all steps leading away.

Numerical integration sets up the appropriate differential equations: i.e.,

$$[X_i]_{t+\Delta t} = [X_i]_t + [(dX_i)/dt]\Delta t$$

Δt must be small enough to keep the solution of the equation within certain bounds and computers must be fast enough to allow long time simulations.

The full time course of a reaction

Kintek Explorer - Student Version

Google Kintek Explorer and open Kintek Explorer Global Simulation Software

Go to the bottom of the page and Download KinTek Explorer Student for your computer

After expanding you will find the program under _kin

You can read the manual or just be aware of a few instructions. Because this is the student version you cannot create a new mechanism or save data. That's the professional version that costs $$.

The mechanism that appears is E + S == ES == EA and the rate constants used are given under the section called Reactions. By placing the mouse over one of the numbers, you can change the rate constant and watch in real time the change in the time course of the reaction. You can fix rate constants to a certain value by clicking on the button to turn it red. Assume the rate constants are in sec^{-1}, μM^{-1}. Do you think the value of 0.13 is appropriate?

For this example, the different colors in the graph show different substrate concentrations.

In the Experiment Editor, chose just one substrate concentration rather than four.

At the bottom of this window is a section called Observables. In this case the observable is ES times a factor plus a second factor.

Add an observable for EA.

Find which rate constant(s) you can change to change the lag for the formation of EA

Under Model Editor, click on Open. You will find a number of possible mechanisms. Choose one and click on OK at the bottom.

As your problem set, discuss the one you choose and how changing specific rate constants affects the time course.

Allostery for 1 and 2 substrates

Monod et al $E_n \rightleftharpoons E_n'$; $L = \frac{\text{loose}}{\text{tight}} = \frac{T}{R} = \frac{E_n'}{E_n}$

Minimal model

$$\bar{Y} = \frac{\alpha(1+\alpha)^{n-1} + L c \alpha (1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$

where α = reduced substrate conc'n = S/K_1

c = ratio of dissociation constants $0 < c < 1$

For kinetics, minimal assumption

$$v_0 = \frac{k_s \alpha (1+\alpha)^{n-1} + k_t L c \alpha (1+c\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\alpha)^n}$$

where k_s = $ES \rightarrow E + P$ } are rate limiting steps

k_t = $E'S \rightarrow E' + P$ }

If $k_s = 0$, get substrate inhibition

Effect of activator or inhibitor is to change L

$$L = L' \left\{ \frac{1+\mu}{1+d\mu} \right\}^n$$

where μ = reduced modifier conc'n = $(M)/K$

and d = ratio of dissociation constants for modifier binding

$d < 1$, modifier binds preferentially to E_n' (forms T that binds S loosely)

$d > 1$, M binds preferentially to E_n

The two substrate case:

The kinetic equation for allosteric behavior, n subunits and 2 conformational forms

$$\frac{\alpha\beta(1+\alpha+\beta+\alpha\beta)^{n-1}+\ Lcd\alpha\beta(1+c\alpha+d\beta+cd\alpha\beta)^{n-1}}{(1+\alpha+\beta+\alpha\beta)^{n}+L(1+c\alpha+d\beta+\ cd\alpha\beta)^{n}}$$

assuming the binding one substrate does not affect the binding of the other. C and d represent relative binding of A and B to the different forms.

The binding expression making the same assumptions as above as well as assuming multiple (n) subunits in the protein

$$\frac{(\alpha+\beta+2\alpha\beta)(1+\alpha)^{n-1}(1+\beta)^{n-1}+L(c\alpha+d\beta+2cd\alpha\beta)(1+c\alpha)^{n-1}(1+d\beta)^{n-1}}{(1+\alpha)^{n}(1+\beta)^{n}+L(1+c\alpha)^{n}(1+d\beta)^{n}}$$

If L=0 (or infinity) and n=1, both equations reduce to the simple one site case.

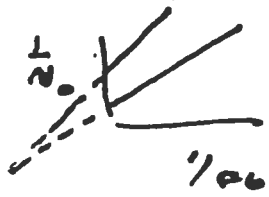
PFK (phosphofructokinase) Example of complex allosteric behavior - Muscle enzyme

Reaction

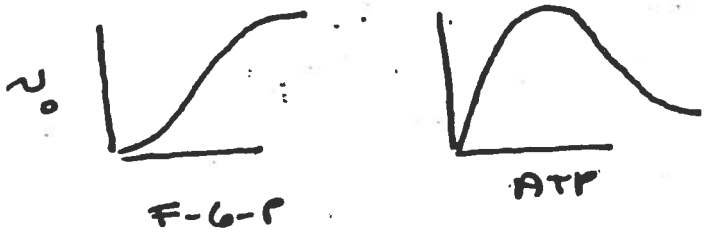
$$F\text{-}6\text{-}P + ATP \xrightarrow{(Mg)} F1,6\,P_2 + ADP + H^+$$

Assay: coupled enzyme system
PK
LDH
FDPase

Behavior at pH 8 - Normal kinetics

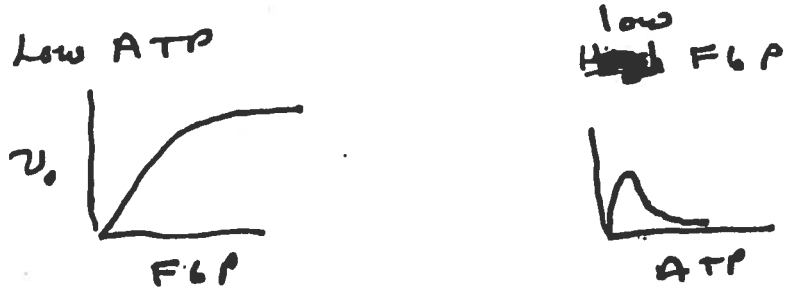


Behavior at pH 6.9



but cooperativity extent depends on [ATP]

and ATP inhibition depends on [F6P]

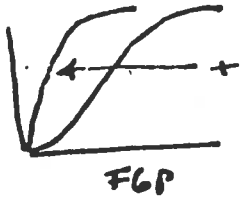


PFK. 2

Effectors – None at pH 8.0

At pH 6.9

F 1,6 P_2 – would expect to be product inhibitor, but activates



other activators AMP, cAMP, ADP, NH_4^+

Inhibitors

ATP (binding to a non-active site)
Citrate

Further complications:

pH dependent tetramer ⇌ dimer

tetramer – active
dimer – inactive.

Therefore – two mechanisms of control

- allosteric – rapid
- association-dissociation could be slow

Both are pH dependent.

PFK-3

Some ways to explain the data

1) Monod-like mechanism where the effector is H^+. At high pH, a single form of the enzyme shows normal behavior. At lower pH values, two forms that differ in binding affinity for substrates. The protonated form is inactive, but binds F-6-P preferentially

2) There is an second inhibitory site for ATP and another site that can bind activators like cAMP or ADP

3) Clearly another site for F-6-P.

4) The tetrameric active enzyme reversibly dissociates to an inactive dimer at lower pH values. This process is effected by substrates - ATP favors dissociation, F-6-P favors association.

More allostery

Although the term allosteric enzymes was originally applied to multisubunit proteins, it has become a catch-all term for any system where sites distant from the active site affect enzymatic behavior. Thus, it applies to enzymes that are monomeric.

Example: the protease thrombin.

A protein of mol wt 36k it is a part of the coagulation cascade converting (among other things) fibrinogen to fibrin. See J Biol Chem (2004) *279* 31842-31853 Like all trypsin-like proteases, thrombin has the three catalytic residues: His57, Asp102 and Ser195. But there is a Na^+ binding site that is distinct from the active site that is involved with substrate recognition.

The results of the paper sited above identify structural determinants responsible for the Na^+ dependent enhancement of catalytic activity. The changes are small but critical. Two forms are defined – fast and slow. The figure show the structural changes induced by Na^+ binding

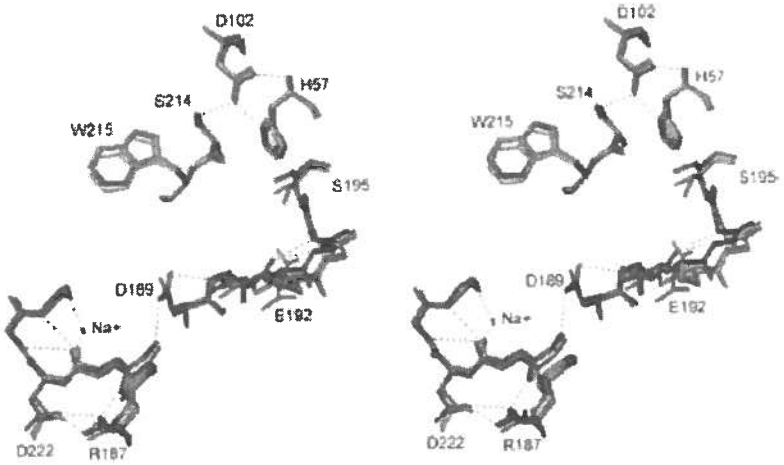
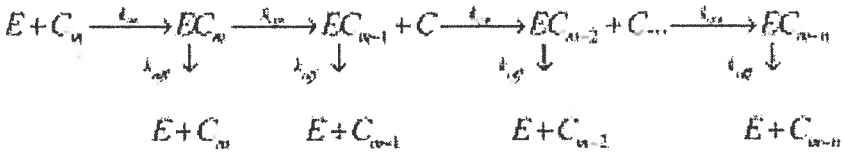


FIG. 6. **Stereo view of the structural changes induced by Na^+ and/or PPACK binding to thrombin.** Structures depict **F** (free fast form, *gold*), **S** (free slow form, *red*), **FL** (PPACK-bound fast form, *blue*), and **SL** (PPACK-bound slow form, *green*). The bound PPACK in the **SL** and **FL** structures was omitted to expose the changes at the level of Ser-195. Shown are the changes in the Arg-221a backbone (**S**), the breakage of the Arg-187:Asp-222 ion pair (**S**), the reorientation of Asp-189 (**S**), the upward shift of the 191–193 strand (**SL** and **FL**), the reorientation of Glu-192 (**S** and **F**), and the shift in the position of the O_γ atom of Ser-195 (**S** and **F**). H-bonds are shown by *broken lines* and refer to the **FL** structure.

Processivity is the ability of an enzyme to catalyze many consecutive reactions prior to dissociation of the enzyme from the substrate. Polymerases, helicases are assemblies of subunits and the mechanism of action is complex. However, these enzymes do dissociate from their substrates at some point. One can imagine, for example, an inchworm type mechanism where the enzyme moves ahead to grasp a new region of the substrate while dissociating from the previous region.
What are the kinetics of processive enzymes. For any substrate that has repeating structures: helicases, polymerases, cellulases etc

Statistical kinetics of processive enzymes
From Cruys-Bagger et al. (2013) Biochemistry 52 8938-8948

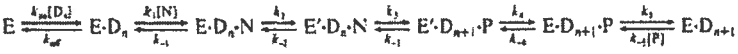
Scheme 1. Simplified Reaction Scheme for the Hydrolysis of a Cellulose Strand by a Processive Enzyme[a]



[a] The enzyme, E, attacks the end of a strand, C_m, with m cellobiose units and moves processively along the strand. Cellobiose, C, is released and the strand is sequentially shortened to C_{m-1}, C_{m-2}, and so forth. The reaction is governed by four parameters: rate constants for association (i.e., formation of an activated complex), catalysis, and dissociation and a processivity number, n.

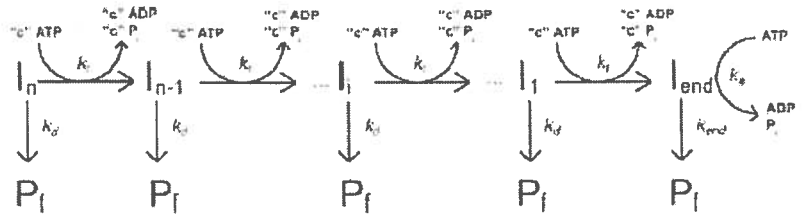
From Patel et al. (1991) Biochemistry 30 511-525

ABSTRACT: The elementary steps of DNA polymerization catalyzed by T7 DNA polymerase have been resolved by transient-state analysis of single nucleotide incorporation, leading to the complete pathway:



where E, D, N, and P represent T7 DNA polymerase, DNA primer/template, deoxynucleoside triphosphate, and inorganic pyrophosphate, respectively. A DNA primer/template consisting of a synthetic 25/36-mer

From Fischer et al. Methods in Molecular Biology 875 (2012)



Scheme 4.1