An Introduction to Enzyme and Coenzyme Chemistry, 3rd Ed.
T. D. H. Bugg, Wiley, New York, 2012

Hydrolytic Enzymes:

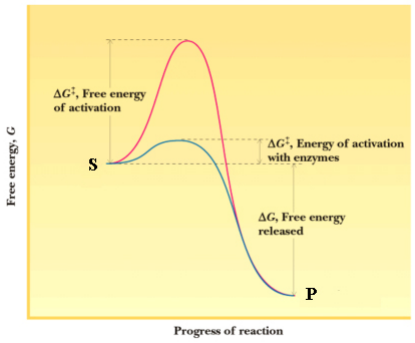
Proteases	Chapter 5, pp 79-92
Phosphatases	Chapter 5, pp 95-102

Cofactors (Coenzymes):

redox	2 e^-	Thiamin	Chapter 7, pp 165-188
		Pyradoxal phosphate	Chapter 9, pp 197-212
		Nicotinamide	Chapter 6, pp 115-122
	1 e^- or 2 e^-	Flavin Coenzymes	Chapter 6, pp 122-133
mutase	1 e^-	Heme	Chapter 6, pp 136-140
		Vitamin B_{12}	Chapter 11, pp 225-229

139

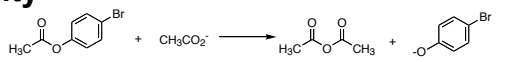
Enzymes:
all enzymes are proteins
catalysts speed up reactions by lowering the activation energy ($\Delta G^{\ddagger}$), NOT by changing the thermodynamics of the reaction ($\Delta G°$)

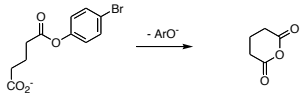


Stabilization of the transition state
Bringing reactants together in the proper orientation for the reaction to occur

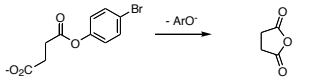
140

Proximity

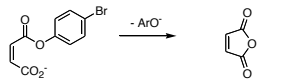




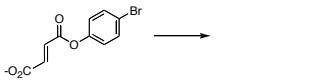
K_{rel} = 220 s^{-1} effect molarity 220 M

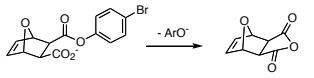


K_{rel} = 5 x 10^4 s^{-1} effect molarity 5 x 10^4 M



K_{rel} = 2 x 10^6 s^{-1} effect molarity 2 x 10^6 M

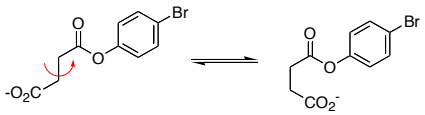




K_{rel} = 1 x 10^7 s^{-1} effect molarity 1 x 10^7 M

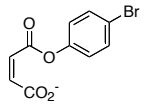
141

Bugg, pp 30-32



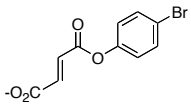
K_{rel} = 5 x 10^4 s^{-1}

reactive conformation for cyclization reaction



held (pre-organized) in the reactive conformation

K_{rel} = 2 x 10^6 s^{-1}



142

Mechanism:

Mechanisms can not be proven absolutely, they can only be disproved (inconsistent with the available data)

Accepted mechanism is based on the preponderance of the available evidence

- consistent with the products - labeling studies
- intuition: consistent with well-known chemistry and widely accepted mechanistic tenets
- consistent with modest changes in the substrate
- kinetic rate expression
- model reactions - easier to study

143

Enzyme Commission (EC) classification – IUBMB
#.##.##.X##

Class	Reaction
EC 1	**Oxidoreductases** – calatyzes oxidations and reductions
EC 2	**Transferases** – functional group transfer
EC 3	**Hydrolases** – hydrolysis (overall addition of H_2O) to a substrate to give two products
EC 4	**Lyases** – non-hydrolytic addition of removal of groups (i.e., H_2O, NH_3, etc) from a substrate.
EC 5	**Isomerases** (mutase) – product is a structural isomer of the substrate
EC 6	**Ligases** – joins two substrates by a bond formation reaction of two substrates using ATP to drive the reaction.

EC#'s define a biochemical reaction, not a specific enzyme

http://www.brenda-enzymes.org/index.php4

144

Simple enzymes: consists only of the proteins.
Complex Enzyme: Protein contains other non-protein group(s) that assistant in the catalysis = coenzyme (vitamins or metal ions)

Coenzymes are bound to the protein by non-covalent interactions
Prosthetic group: cofactor is covalently bound to the protein

Holoenzyme – protein – coenzyme complex
Apoenzyme – protein only, missing the coenzyme.

145

Rates, equilibria and reaction order
Velocity: $v = k\,[S]$

$$S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} P$$

$v_1 = k_1[S]$
$v_{-1} = k_{-1}[P]$

$$K_{eq} = \frac{[P]}{[S]} = \frac{k_1}{k_{-1}}$$

Reaction Order:

$S \longrightarrow P$	first order reaction	$v = k\,[S]$
$S_A + S_B \longrightarrow P$	second order reaction	$v = k\,[S_A]\,[S_B]$

half-life $t_{½} = \ln 2/k$ (k = first order rate constant)

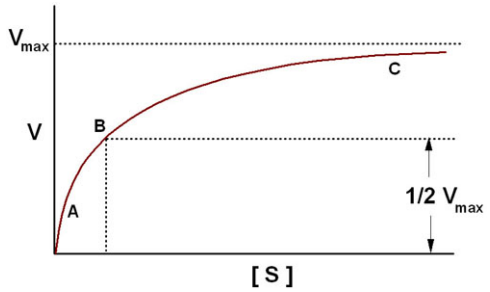
Rate-limiting (determining) step: slowest step in a multi-step chemical reaction. The overall rate of a reaction is dependent on the rate-limiting step

146

Bugg, pp 52-59

Plot of substrate concentration versus reaction velocity

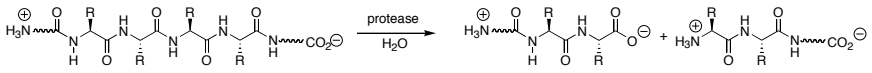
$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [S \bullet E] \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P + E$$



Steady-state approximation: [S•E] is constant
V_{max} : the maximum velocity at saturating [S]
K_m : [S] at half the V_{max}; rough estimate of the affinity of the S
k_{cat} : V_{max}/[E] ; turnover number
V_{max}/K_m or ***k_{cat}/K_m*** **:** catalytic efficiency of the reaction

147

Proteases: catalyzes the hydrolysis of peptide bonds
Bugg, Chapter 5, pp. 81-98



1. Serine protease Bugg, p. 84
2. Cysteine protease p. 89
3. Aspartyl protease p. 95
4. Zinc (metallo) protease p. 92

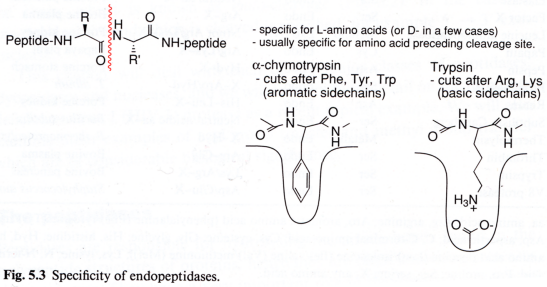
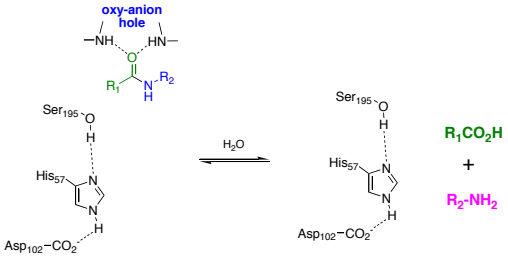


Fig. 5.3 Specificity of endopeptidases.

•chymotrypsin: cleaves at the C-terminal side of aromatic residues Phe, Tyr, Trp
• trypsin: cleaves at the C-terminal side of basic residues Arg, Lys but not His

148

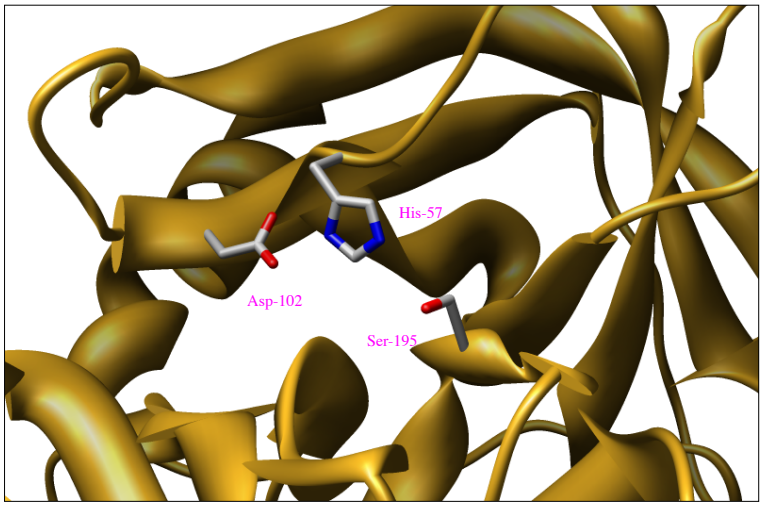
Serine Protease: Chymotrypsin



149

Bugg, p. 82

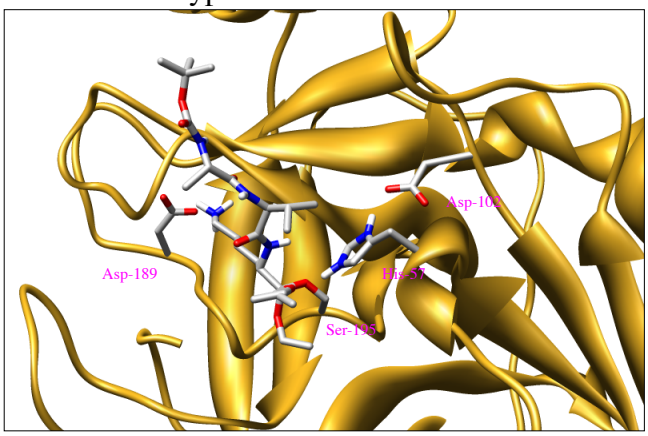
Catalytic triad of α-chymotrypsin

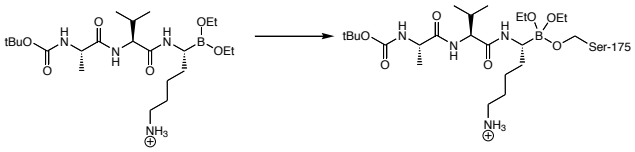


pdb code: 5CHA

150

Active site of bovine trypsin with a bound inhibitor





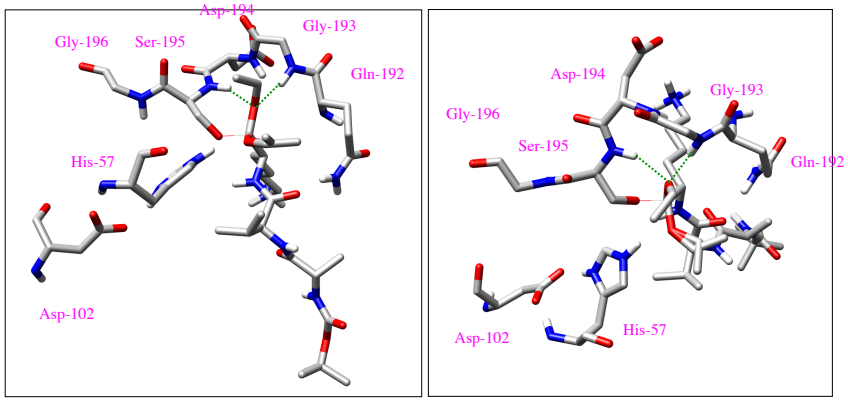
pdb code: 1BTX

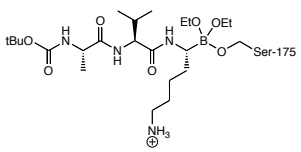
Katz, B. A.; Finer-Moore, J.; Mortezaei, R.; Rich, D. H.; Stroud, R. M. Biochemistry 1995, *34*, 8264-8280.

Bugg, p. 81

151

Oxy-anion hole of trypsin





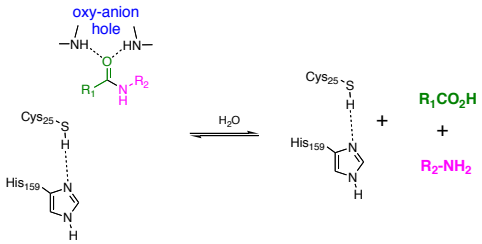
pdb code: 1BTX

152

Cysteine Protease: Papain (212 amino acids)

active-site cysteine and histidine, overall mechanism is similar to serine proteases

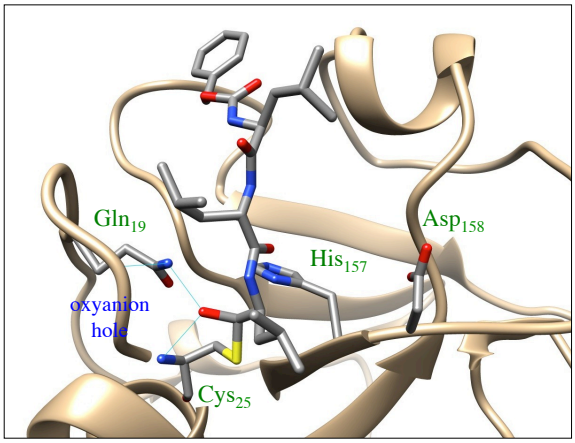
usually not a digestive enzyme (intracellular)

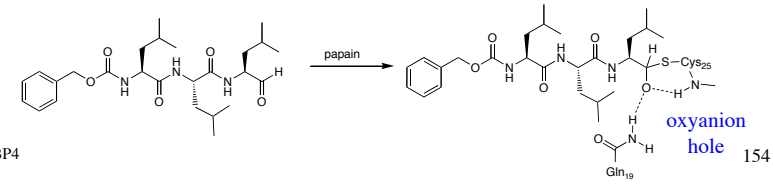


153

Bugg, p. 85

Structure of papain with a bound inhibitor



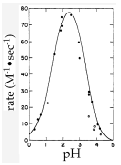


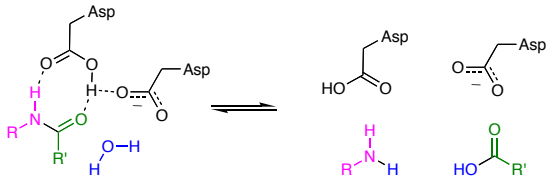
pdb code: 1BP4

154

Aspartyl Protease Mechanism: Renin, HIV protease

Bell shaped pH vs. rate profile: max rate at pH ~ 2.4 indicative of *general acid-general base catalysis*.

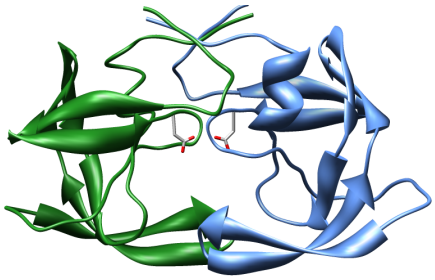




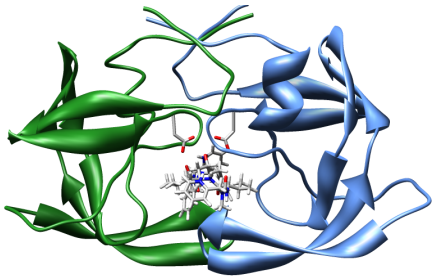
Bugg, p 91

155

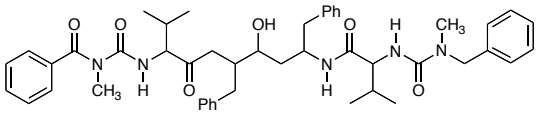
HIV Protease: an Aspartyl Protease



Catalytic Asp-25



with a bound inhibitor

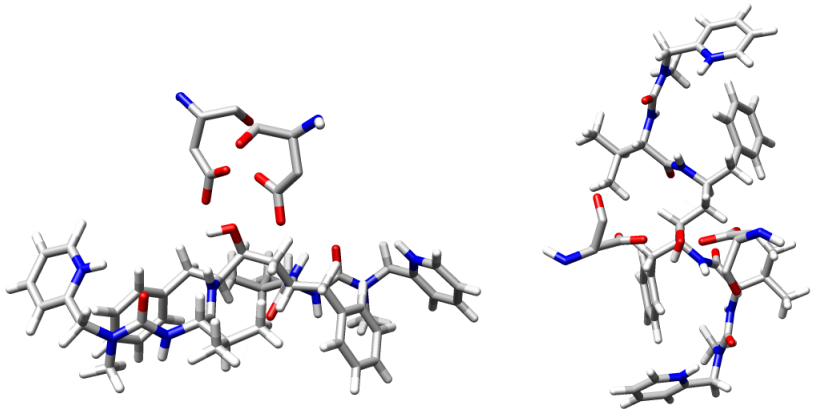


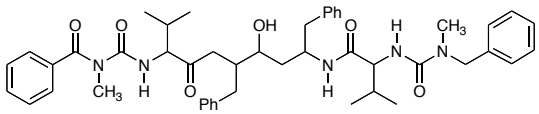
inhibitor

pdb code: 1HVJ

156

Interaction of the catalytic Asp-25 with the bound inhibitor





pdb code: 1HVJ

inhibitor

157

Metallo-protease (zinc): carboxypeptidase:

important catalytic groups: Glu-270, Tyr-248

Zn ion coordination: Glu-72, His-196, His-69

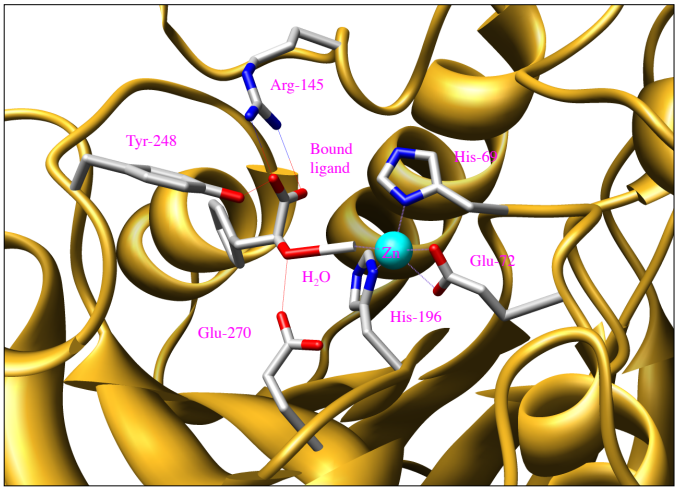
General base mechanism

Nucleophilic mechanism (acyl enzyme complex)

158

Bugg, p. 88

Active site of carboxypeptidase



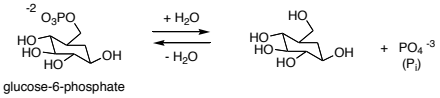
pdb code: 2CTC

159

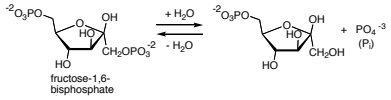
Phosphate ester hydrolysis

1. Phosphatases: over transfer (hydrolysis) of a phosphate monoester to water
2. Phosphodiesterases: hydrolysis of a phosphodiester to a phosphate monoester and an alcohol
3. Kinases: transfer of the γ-phosphate group of ATP to an acceptor group

Glucose 6-phosphatase



Fructose 1,6-bisphosphatase: M^{+2} dependent



Alkaline phosphatase: M^{+2} dependent

160

Bugg, pp. 95-102

Phosphatases: General acid-base mechanism

161

Phosphatases: Covalent mechanism

S_N2 mechanism

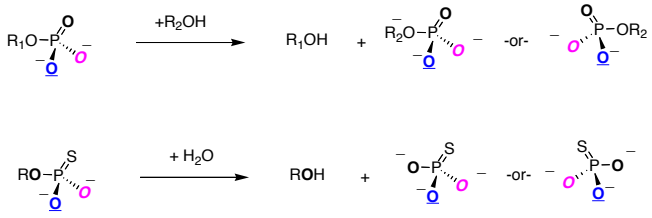
162

Glucose 6-phosphatase: covalent catalysis

Fructose 1,6-bisphosphatase: General acid-base catalysis

163

Associative *vs* dissociative mechanism: Chiral phosphate
Use three isotopes of oxygen, ^{16}O, ^{17}O, ^{18}O

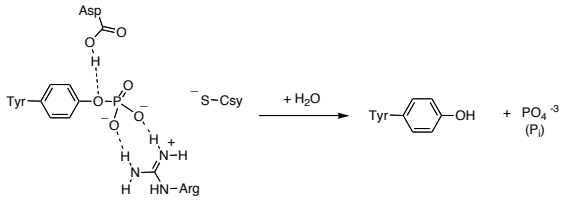


Does the observation of stereochemically defined products (retention or inversion of stereochemistry) rule out the dissociative mechanism involving metaphosphate ion?

164

Tyrosine phosphatase

conserved aspartate, cysteine, and arginine

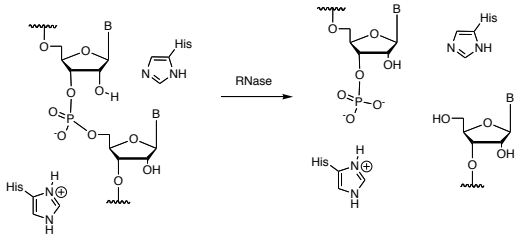


165

Phosphodiesterase:

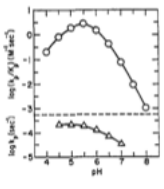
Ribonuclease (RNase): catalyzes the hydrolysis of the phosphodiester bond of RNA.

Catalytic groups are His-12 and His-119



Bell shaped pH vs. rate profile: max rate at pH ~ 6.0 indicative of general acid-general base catalysis.

Mechanism requires both an imidazole and imdiazolium for catalysis

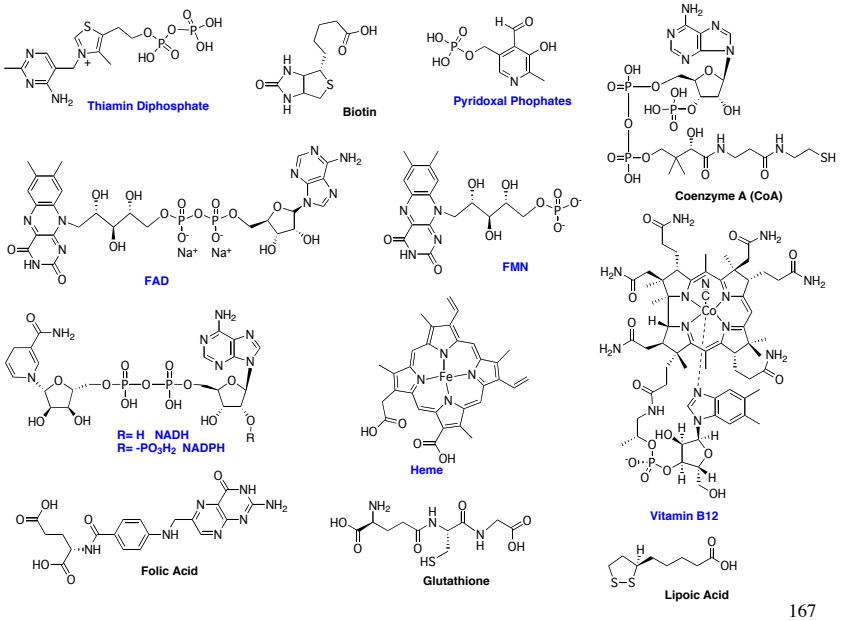


pK_{a3} of hisidine is ~ 6.1

166

Bugg, pp. 99-100

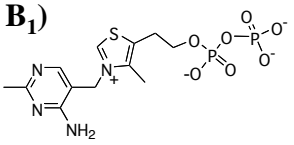
Some Cofactors



167

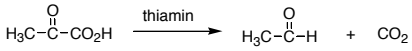
Thiamin Dependent Reactions (Vitamin B_1)

(Bugg, Chapter 7, pp. 165-8)

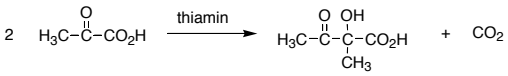


Thiamin Diphosphate (pyrophosphate)

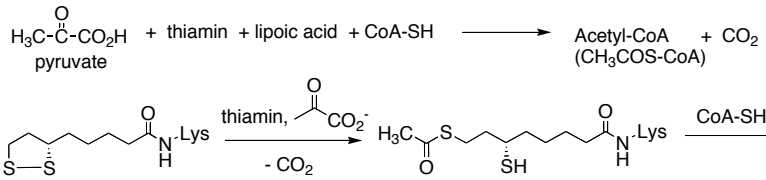
Pyruvate Decarboxylase



Acetolactate synthase



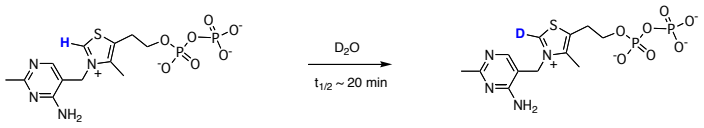
Pyruvate dehydrogenase: pyruvate decarboxylase, dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase,



168

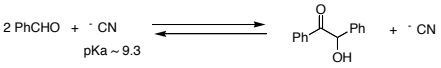
Mechanistic insight into thiamin dependent reactions:

Breslow, R. *J. Am. Chem. Soc.* **1958**, *80*, 3719.



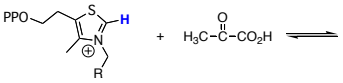
pK_a measured to be between 12-18

Benzoin condensation



169

Mechanism of pyruvate decarboxylase:



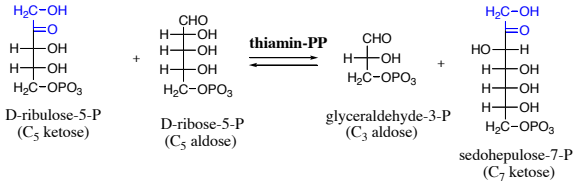
Mechanism of acetolactate synthase:

170

Pyruvate Dehydrogenase: dihydrolipoyl transacetylase

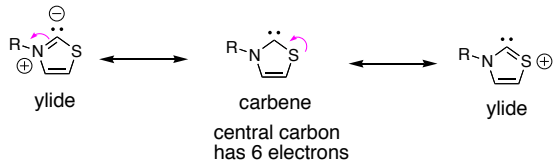
171

Transketolase: carbohydrate biosynthesis

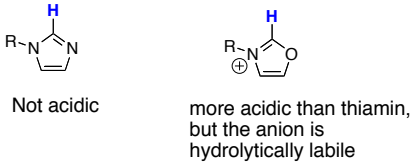


172

Thiamin acidity and anion stability is special to the *N*-alkyl thiazole ring system: anion is stabilized by three major resonance strictures: two are ylides, one is a carbene

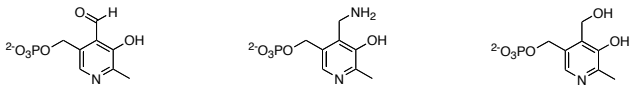


Nature may have tried the imidazole and oxazole ring systems, but these would not have performed the necessary chemistry.

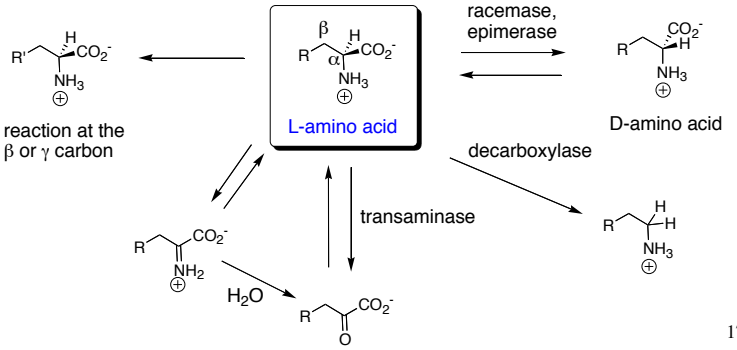


173

Pyridoxal Phosphate (PLP) dependent enzymes (Vitamin B_6)
(Bugg, Chapter 9, pp 197-212)

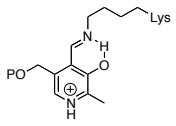


Involved in amino acid biosynthesis, metabolism and catabolism



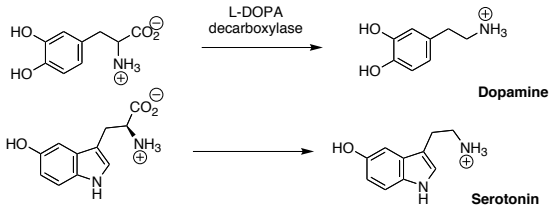
174

Pyridoxal is often covalently bound to the resting enzyme as a Schiff base to the sidechain of an active site lysine residue.



175

Decarboxylase:

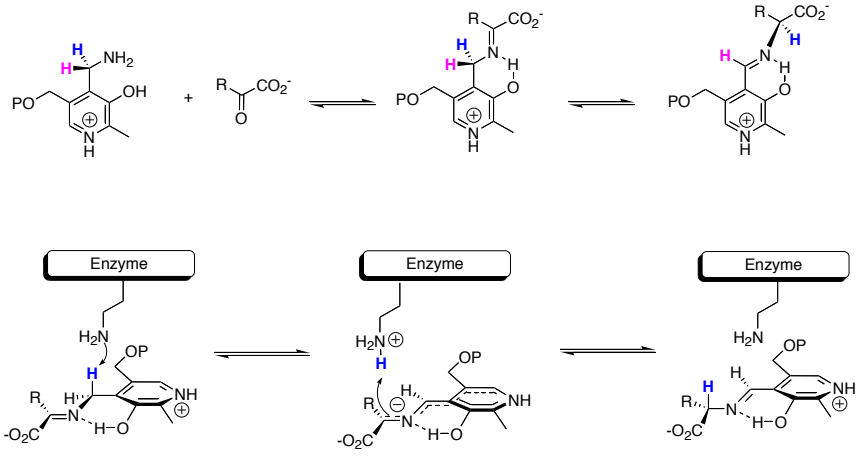


176

Transaminase: amino acid biosynthesis

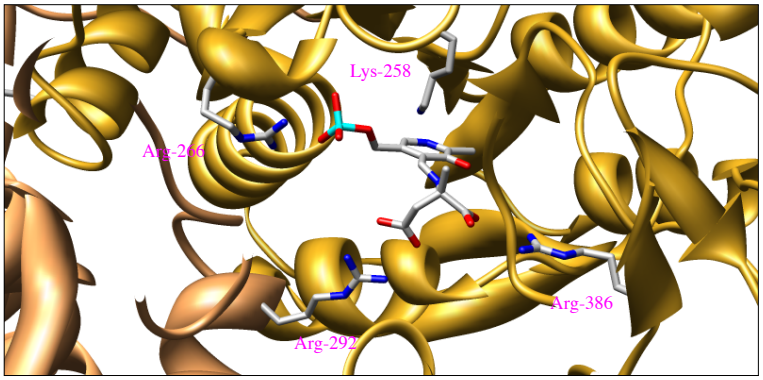
177

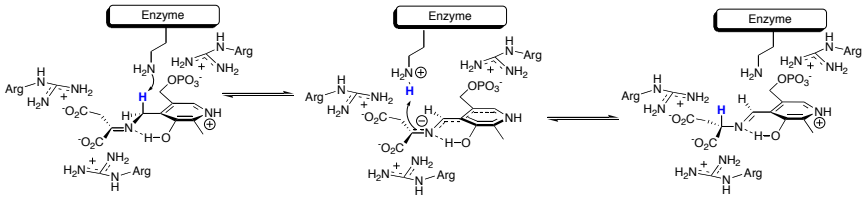
Stereochemistry of the transamination reaction: proton transfer is mediated by an active site lysine



178

Stereochemistry of transamination (aspartate aminotransferase)

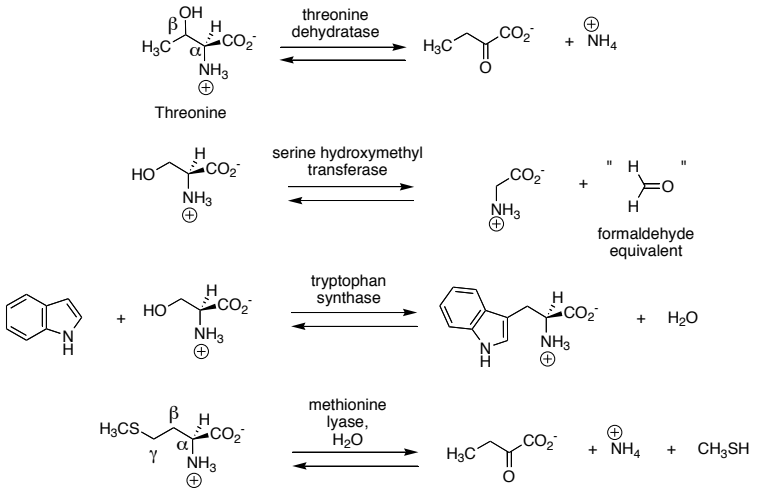




179

Pdb code: 1AJS

Reactions at the β- and γ-carbons of amino acids



180

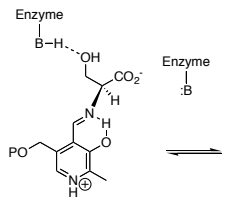
Threonine Dehydratase

181

Tryptophan Synthase:

reactivity of indole toward electrophiles:

Similar to the mechanism of threonine dehyratase an electophilic PAL-amino acid complex is generated



182

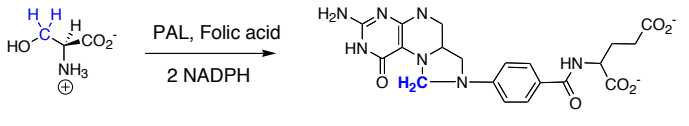
Tryptophan Synthase (con't):

183

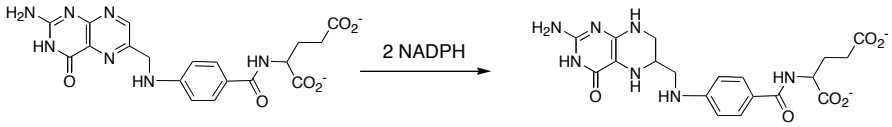
Serine hydroxymethyl transferase:

Bugg, Ch. 9, pp. 206-7

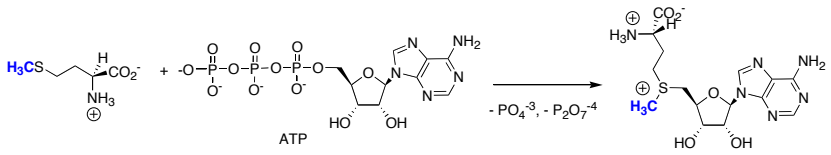
one-carbon (methyl) donors in biology



methylene tetrahydrofolate



Folic acid

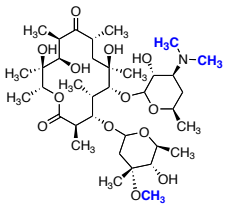


S-adenosyl methionine (SAM)

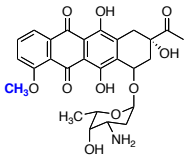
184

Methyl groups from:

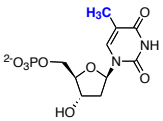
SAM **Methylene tetrahydrofolate**

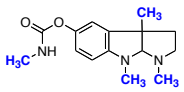


Erythromycin



Daunomycin





Physostigmine

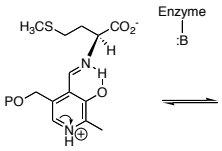
185

Mechanism of serine hydroxymethyl transferase:

Note: this mechanism is not the same as on page 207 of Bugg, which is probably incorrect

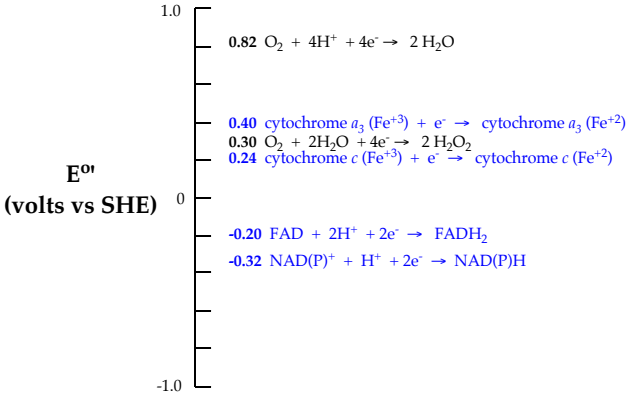
186

Mechanism of methionine γ-lyase



187

Redox Cofactors



Bugg, Chapter 6

188