

An Introduction to Enzyme and Coenzyme Chemistry, 2nd Ed.
T. D. H. Bugg, Blackwell Science, Oxford, 2004

Hydrolytic Enzymes:

Proteases	Chapter 5, pp 81-98
Phosphatases	Chapter 5, pp 102-109

Cofactors (Coenzymes):

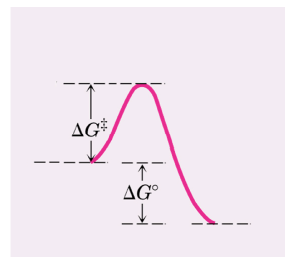
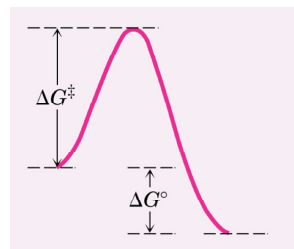
redox mutase	[	2 e ⁻	Thiamin	Chapter 7, pp 176-179
		1 e ⁻ or 2 e ⁻	Pyridoxal phosphate	Chapter 9, pp 210-221
			Nicotinamide	Chapter 6, pp 121-129
			Flavin coenzyme	Chapter 6, pp 129-142
			1 e ⁻	Heme
		Vitamin B12	Chapter 11, pp 240-244	

142

Enzymes:

all enzymes are proteins

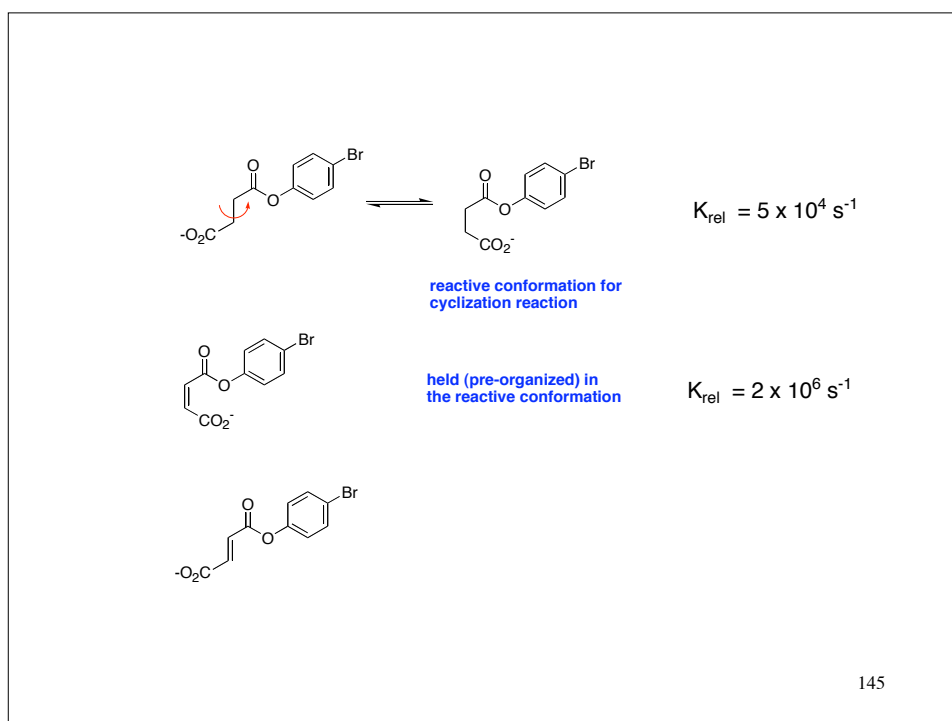
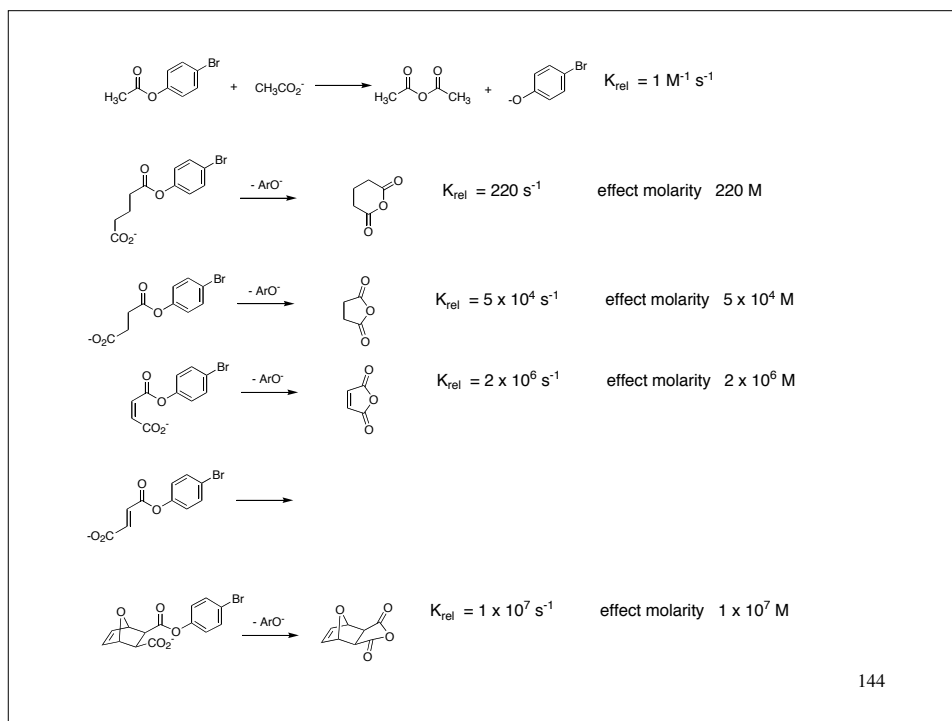
catalysts speed up reactions by lowering the activation energy ($\Delta G^\ddagger$), NOT by changing the thermodynamics of the reaction (ΔG°)



Stabilization of the transition state

Bringing reactants together in the proper orientation for the reaction to occur

143



Mechanism:

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

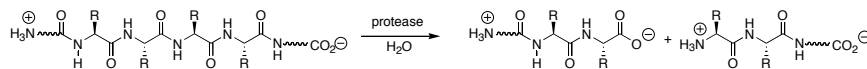
Accepted mechanism is based on the preponderance of the available evidence

- 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

146

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 |

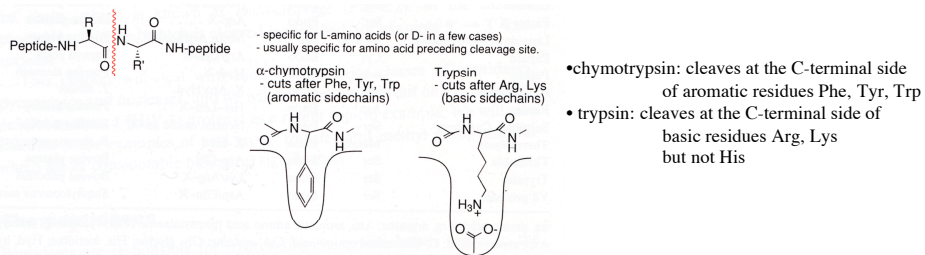
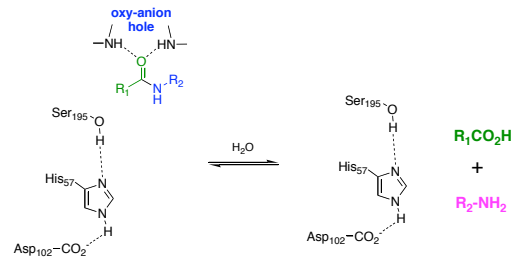


Fig. 5.3 Specificity of endopeptidases.

147

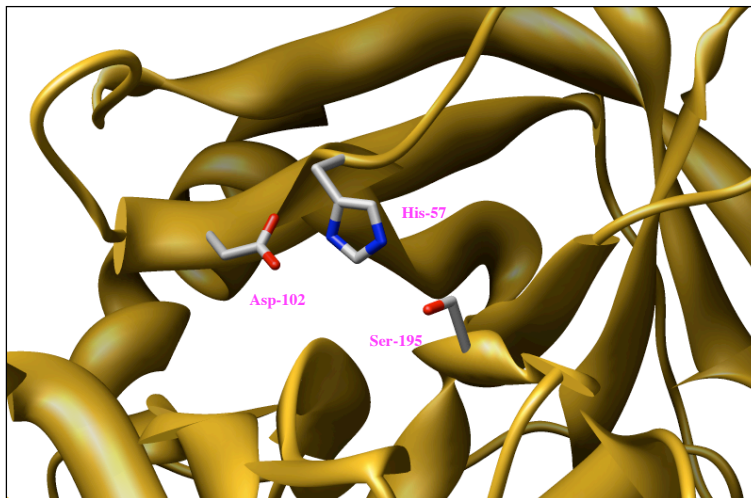
Serine Protease: Chymotrypsin



Bugg, p. 96

148

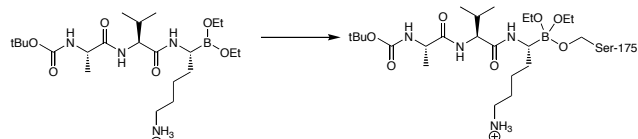
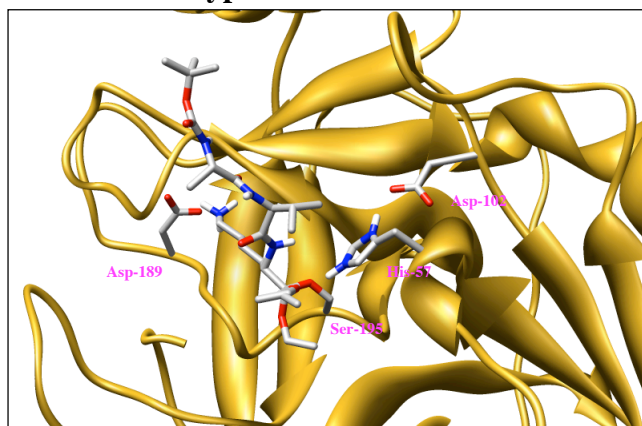
Catalytic triad of α -chymotrypsin



pdb code: 5CHA

149

Active site of bovine trypsin with a bound inhibitor



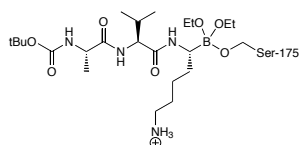
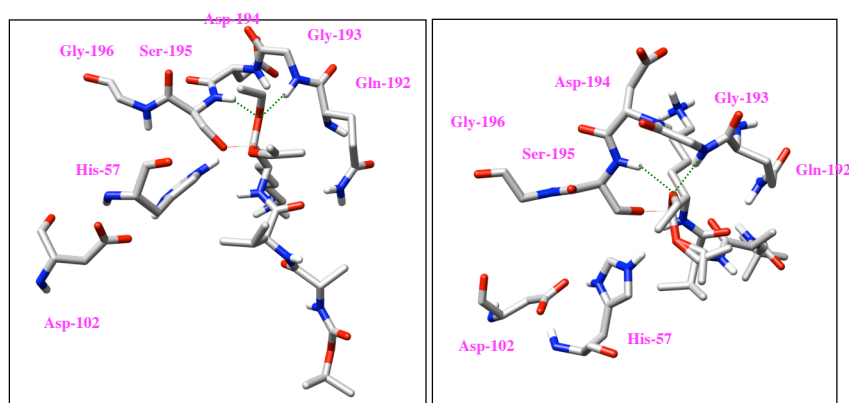
pdb code: 1BTX

Bugg, p. 85

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

150

Oxy-anion hole of trypsin



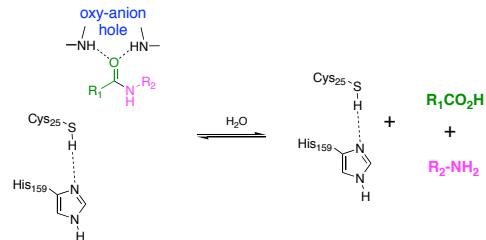
pdb code: 1BTX

151

Cysteine Protease: Papain (212 amino acids)

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

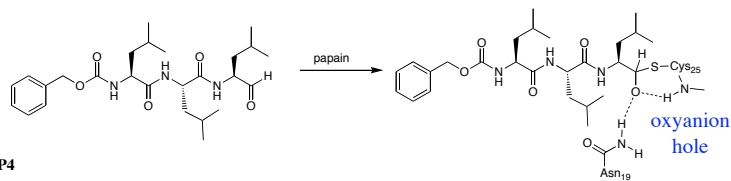
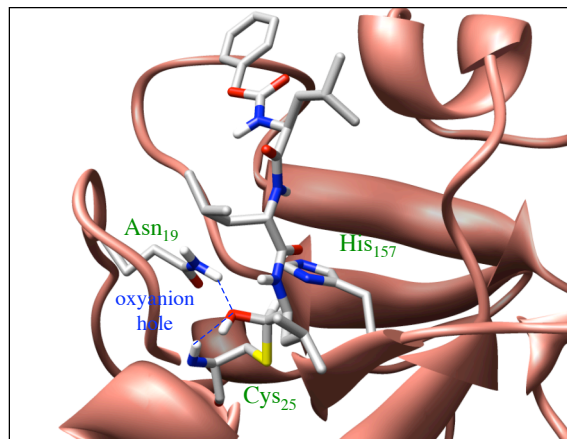
usually not a digestive enzyme (intracellular)



Bugg, p. 91

152

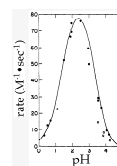
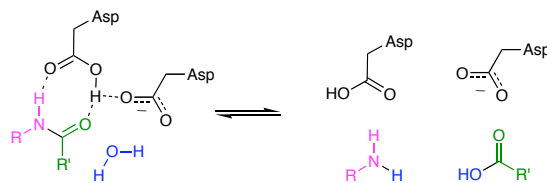
Structure of papain with a bound inhibitor



pdb code: 1BP4

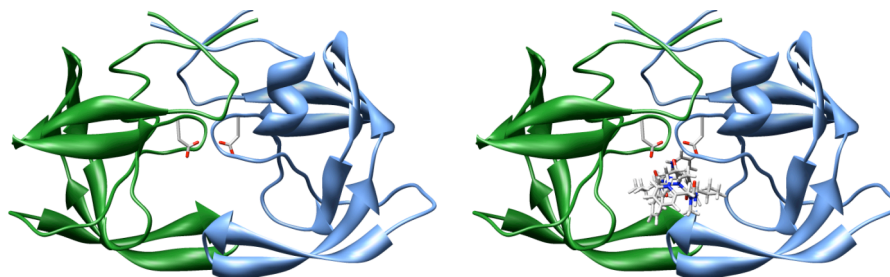
153

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*.



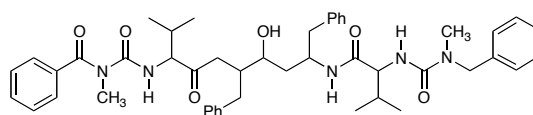
154

HIV Protease: an Aspartyl Protease



Catalytic Asp-25

with a bound inhibitor

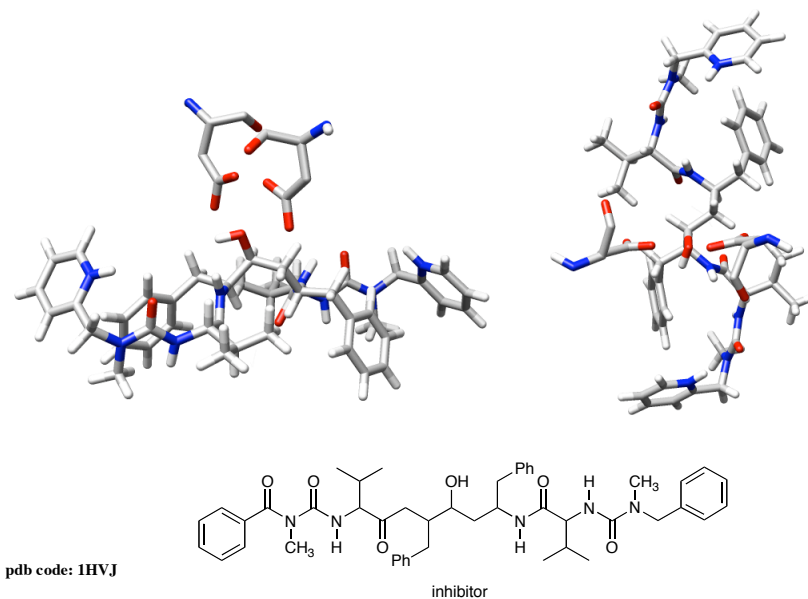


pdb code: 1HVJ

inhibitor

155

Interaction of the catalytic Asp-25 with the bound inhibitor



156

Metallo-protease (zinc): carboxypeptidase:

important catalytic groups: Glu-270, Tyr-248

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

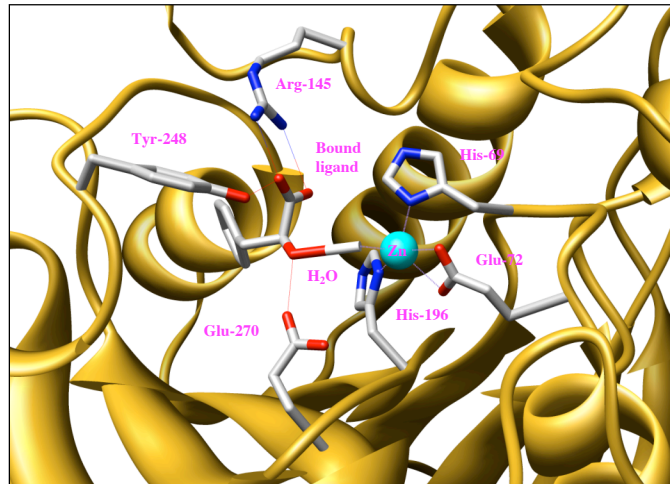
General base mechanism

Nucleophilic mechanism (acyl enzyme complex)

Bugg, p. 94

157

Active site of carboxypeptidase



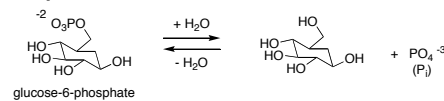
pdb code: 2CTC

158

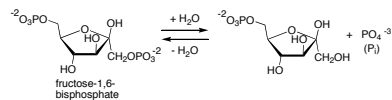
Phosphate ester hydrolysis

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

159

Bugg, pp. 102-109

Phosphatases: General acid-base mechanism

160

Phosphatases: Covalent mechanism

S_N2 mechanism

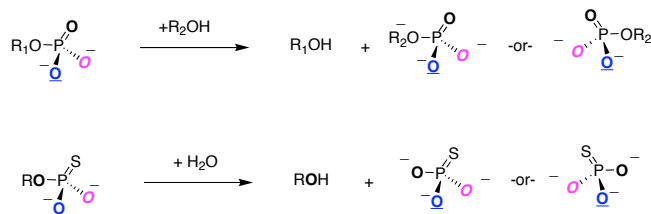
161

Glucose 6-phosphatase: covalent catalysis

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

162

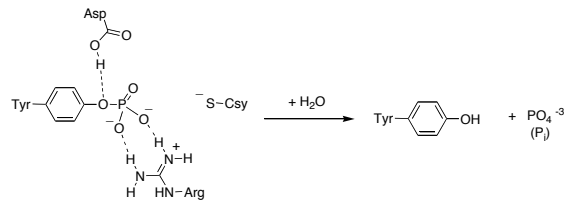
Associative vs dissociative mechanism: Chiral phosphate
Use three isotopes of oxygen, ^{16}O , ^{17}O , ^{18}O



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

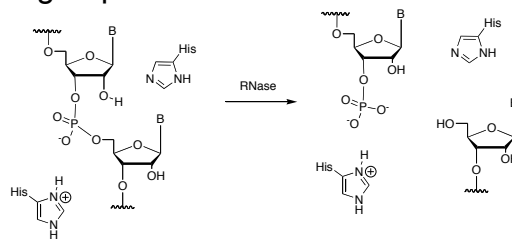
163

Tyrosine phosphatase
conserved aspartate, cysteine, and arginine



164

Phosphodiesterase:
Ribonuclease (RNase): catalyzes the hydrolysis of the phosphodiester bond of RNA.
 Catalytic groups are His-12 and His-119



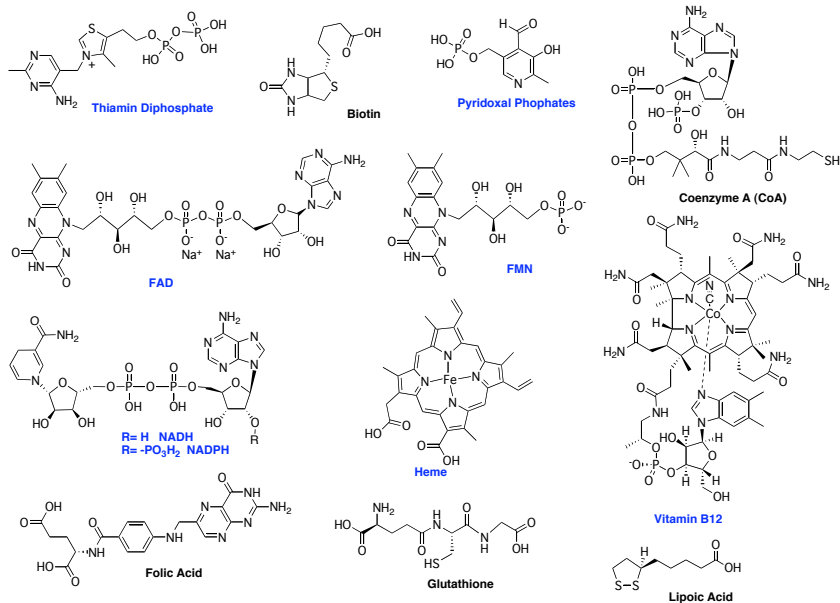
Bell shaped pH vs. rate profile: max rate at pH ~ 6.8 indicative of general acid-general base catalysis. Mechanism requires both an imidazole and imidazolium for catalysis

pKa of histidine is ~ 6.1

165

Bugg, pp. 104-109

Some Cofactors

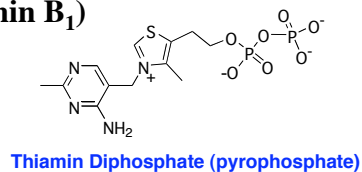
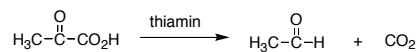


166

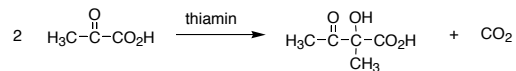
Thiamin Dependent Reactions (Vitamin B₁)

(Bugg, Chapt. 7, pp. 176-9)

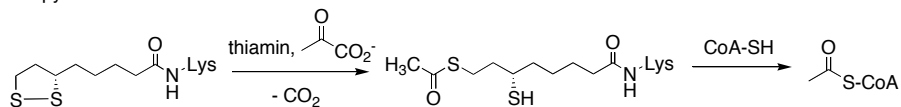
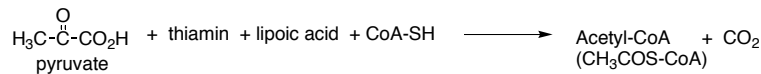
Pyruvate Decarboxylase



Acetolactate synthase



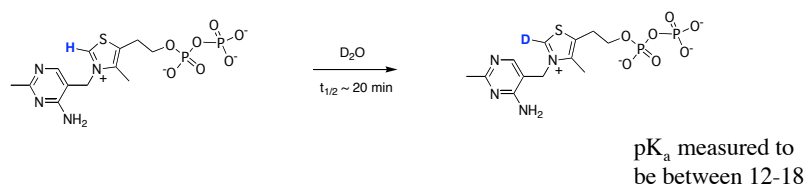
Pyruvate dehydrogenase: pyruvate decarboxylase, dihydrolipoyl transacetylase, dihydrolipoyl dehydrogenase,



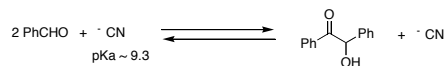
167

Mechanistic insight into thiamin dependent reactions:

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

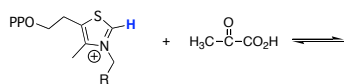


Benzoin condensation



168

Mechanism of pyruvate decarboxylase:



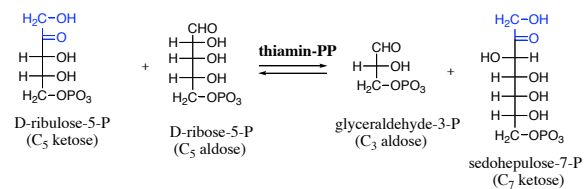
Mechanism of acetolactate synthase:

169

Pyruvate Dehydrogenase: dihydrolipoyl transacetylase

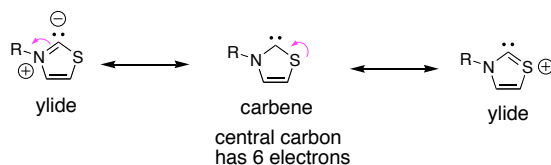
170

Transketolase: carbohydrate biosynthesis

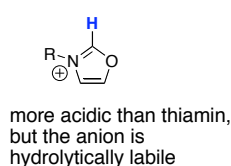
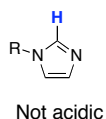


171

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

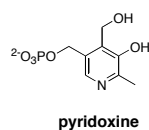
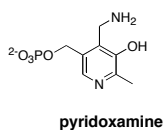
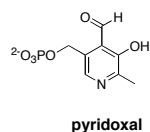


Nature may have tried the imidazole and oxazole ring systems, but these would not have performed the necessary chemistry.

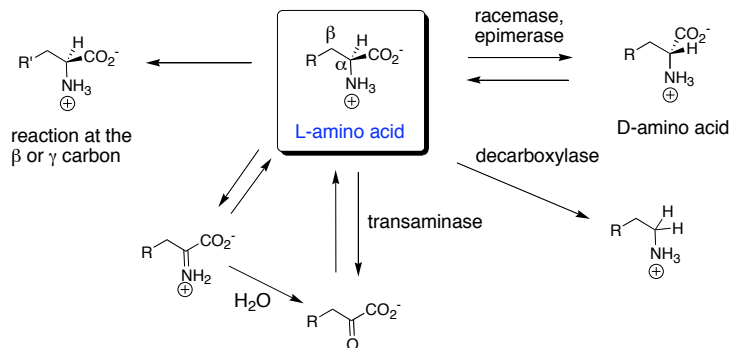


172

Pyridoxal Phosphate (PLP) dependent enzymes (Vitamin B₆) (Bugg, Chapt. 9, pp 210-221)

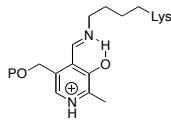


Involved in amino acid biosynthesis, metabolism and catabolism



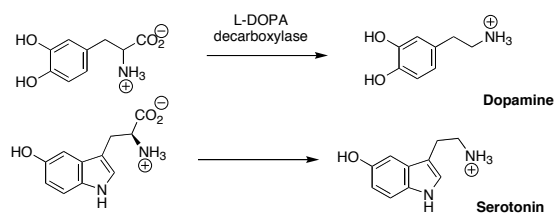
173

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



174

Decarboxylase:

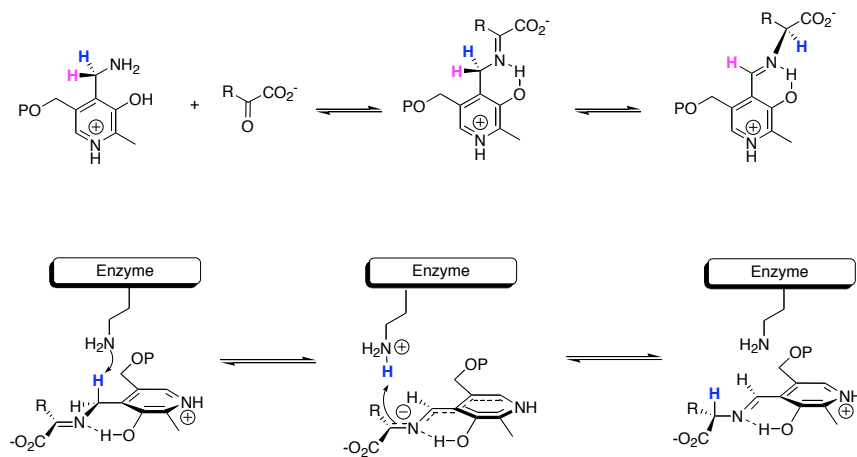


175

Transaminase: amino acid biosynthesis

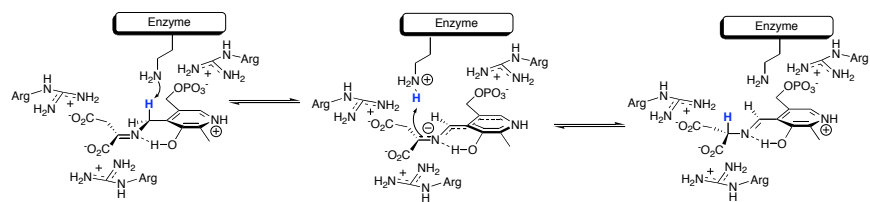
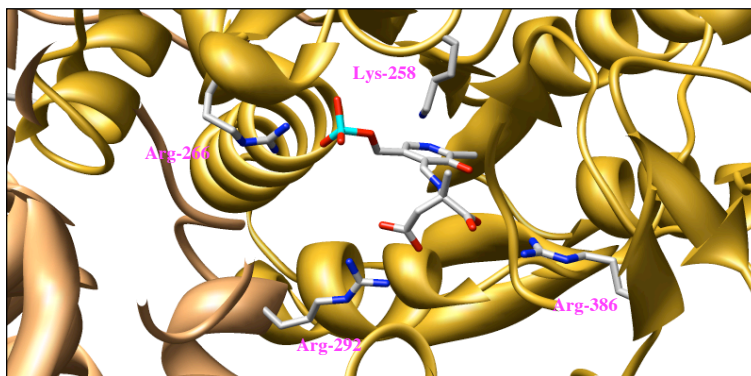
176

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



177

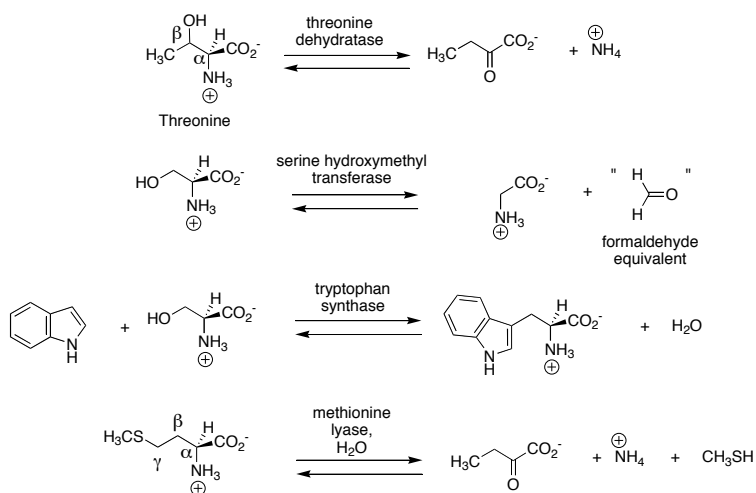
Stereochemistry of transamination (aspartate aminotransferase)



Pdb code: 1AJS

178

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



179

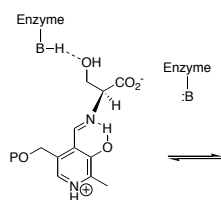
Threonine Dehydratase

180

Tryptophan Synthase:

reactivity of indole toward electrophiles:

Similar to the mechanism of threonine dehydratase an electrophilic
PAL-amino acid complex is generated



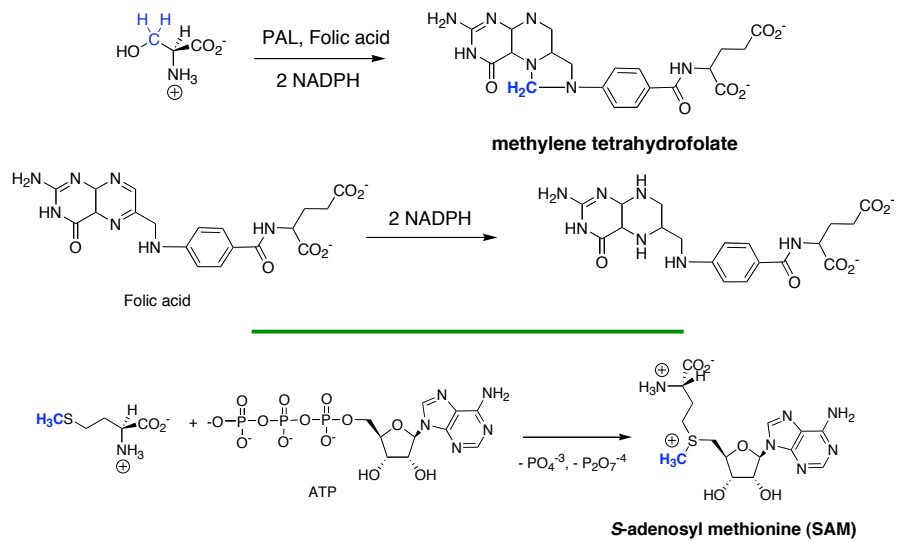
181

Tryptophan Synthase (con't):

182

Serine hydroxymethyl transferase: one-carbon (methyl) donors in biology

Bugg, Ch. 5, pp. 220-1

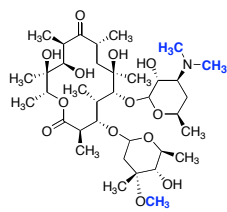


183

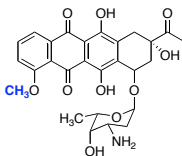
Methyl groups from:

SAM

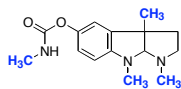
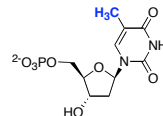
Methylene tetrahydrofolate



Erythromycin



Daunomycin



Physostigmine

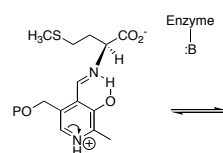
184

Mechanism of serine hydroxymethyl transferase:

Note: this mechanism is not the same as on page 221 of Bugg, which is probably incorrect

185

Mechanism of methionine γ -lyase



186