A Short Introduction to Chemical Biology and Medicinal Chemistry

Part II Ben Davis – 3 Lectures - Enzymes and Their Uses

Learning Outcomes

By the end of this you should be able to

- Compare and Contrast Advantages/Disadvantages of Enzymes as Reagents/Catalysts
- Recall the general mechanism of serine hydrolases
- Compare and contrast this mechanism with other peptide bond making/breaking catalysts
- Recall the mechanism of serine proteases, metalloproteases, aspartylproteases, the ribosome
- Describe examples of uses of acyl transferases in synthesis
- Explain the differing strategies used to accomplish bond making vs bond breaking using acyltransferases
- Give examples of the types of selectivities that may be exhibited by acyl transferases
- Explain the use of enzymes in kinetic resolution (KR), DKR, and desymmetrization
- Recall the mechanisms of glycosidases and glycosyltransferases
- Compare and contrast the mechanism of glycosidases with acyltransferases
- Give examples of methods for protein engineering
- Describe the basic principles behind site-directed mutagenesis
- Compare and contrast site-directed mutagenesis with chemical modification
- Give examples of protein engineering that has altered enzyme reactivity and specificity

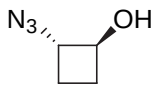
Enzyme movies and X-ray Structures

http://www.chem.ox.ac.uk/researchguide/bgdavis.html

Sample Exam Question Types

1. (a) Give an account of the use of serine hydrolase mechanism enzymes in synthesis [13 marks]
   (b) Comment on the following transformations [5 x 4 marks]
   Biocatalytic reaction e.g.s

2. (a) By giving examples of the way in which enzyme stereoselectivity can be exploited outline 3 complementary methods for obtaining an enantiopure sample of the following:



   [15 marks]

3. (a) The enzyme *chuggase* has the following active site residues and operates on substrate **A** but not substrate **B**. By drawing analogies with classes of enzymes and biocatalysts with which you are familiar propose a mechanism for its catalytic cycle.

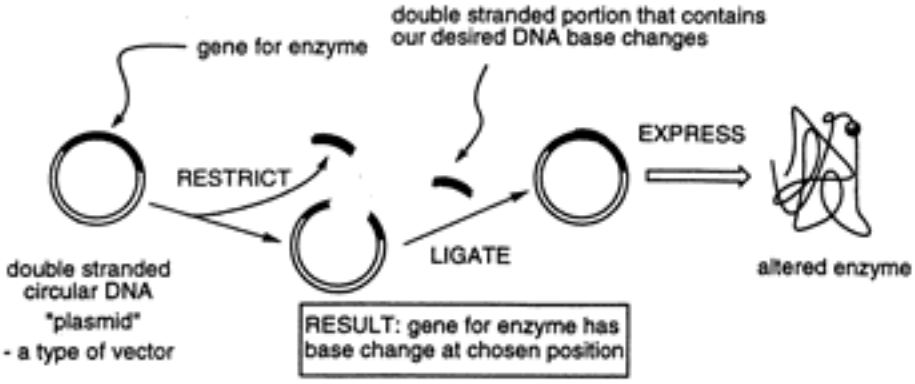
A **B**

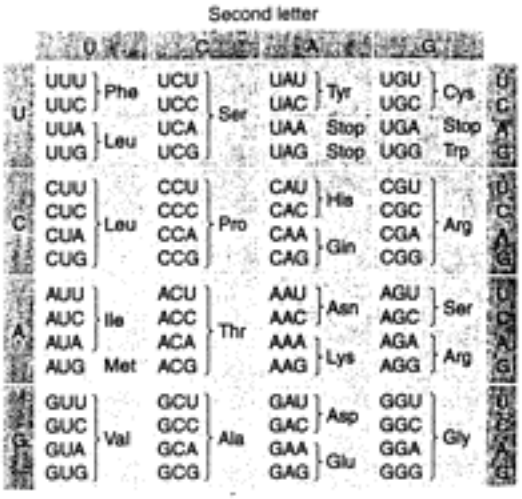
   [15 marks]

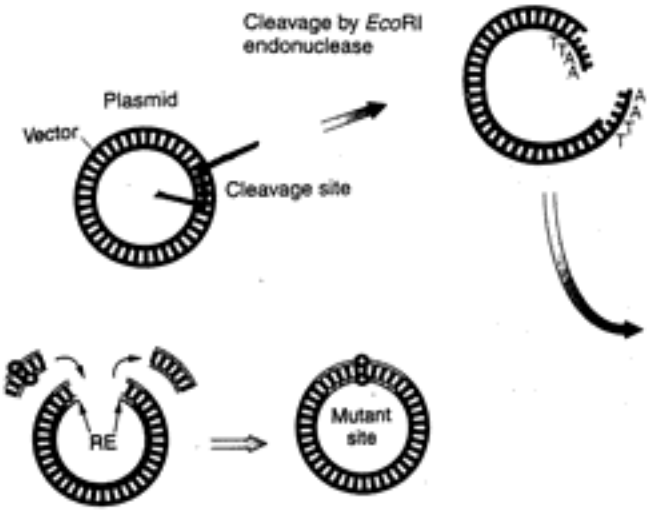
   (b) Given the mechanism that you have proposed, design a *chuggase* inhibitor.

   [10 marks]

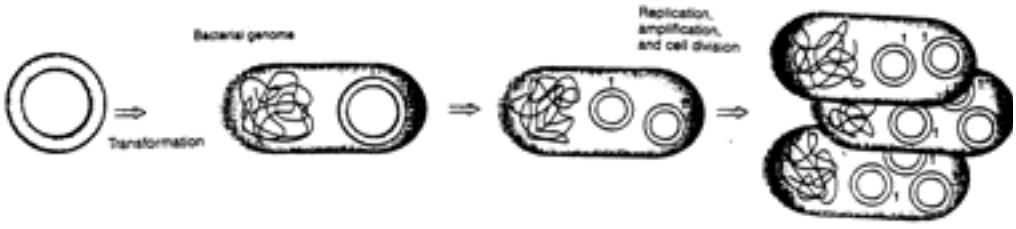
Site-Directed Mutagenesis

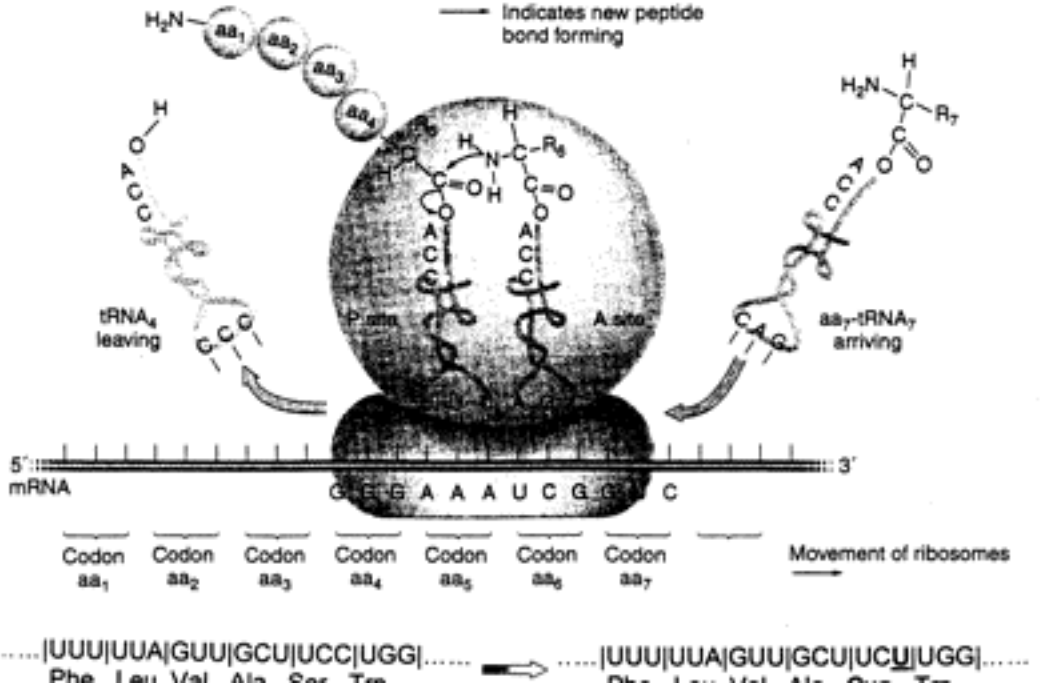






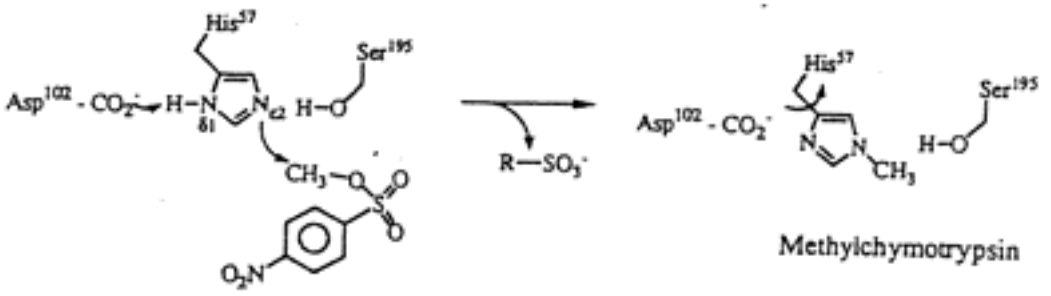
Enzyme	Source organism	Restriction site in double-stranded DNA
EcoRI	*Escherichia coli*	5′ -G-A-A-T-T-C- 3′
EcoRII	*E. coli*	5′ -G-C-C-T-G-G-C- 3′
HindII	*Haemophilus influenzae*	5′ -G-T-Py-Pu-A-C- 3′
HindIII	*H. influenzae*	5′ -A-A-G-C-T-T- 3′
HaeIII	*H. aegyptius*	5′ -G-G-C-C- 3′
HpaII	*H. parainfluenzae*	5′ -C-C-G-G- 3′
PstI	*Providencia stuartii*	5′ -C-T-G-C-A-G- 3′
SmaI	*Serratia marcescens*	5′ -C-C-C-G-G-G- 3′
BamI	*Bacillus amyloliquefaciens*	5′ -G-G-A-T-C-C- 3′
BglII	*B. globiggi*	5′ -A-G-A-T-C-T-



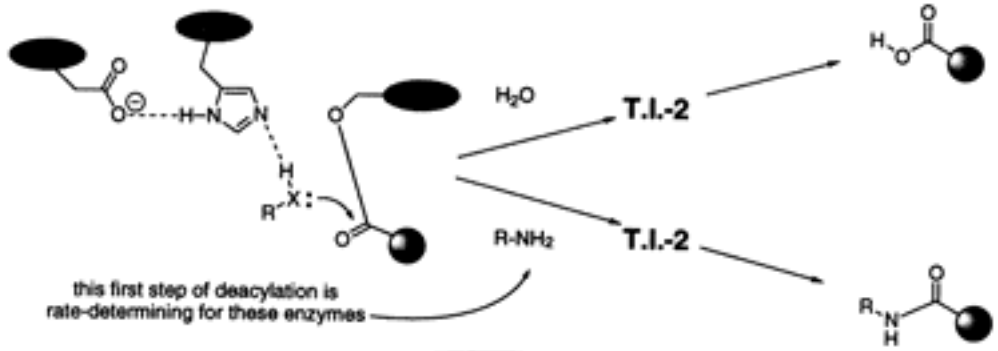


…..|UUU|UUA|GUU|GCU|UCC|UGG|….. ⇒ …..|UUU|UUA|GUU|GCU|UCU|UGG|…..
Phe Leu Val Ala Ser Trp Phe Leu Val Ala Cys Trp

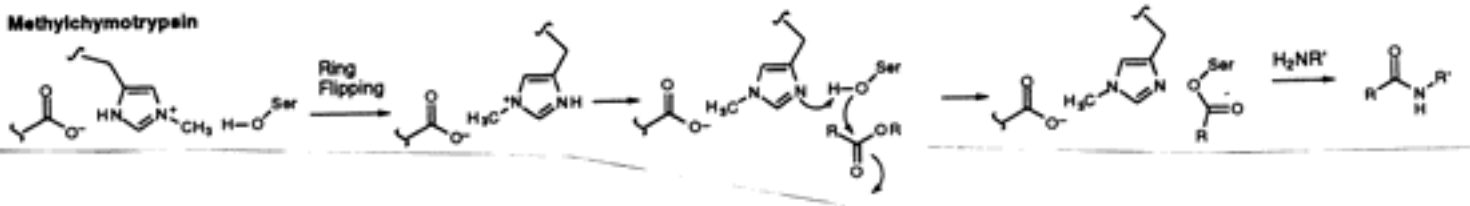
Methylchymotrypsin



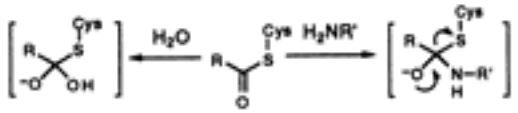
Altered Synthetic Properties of MethylCT and Thio/Selenosubtilisin

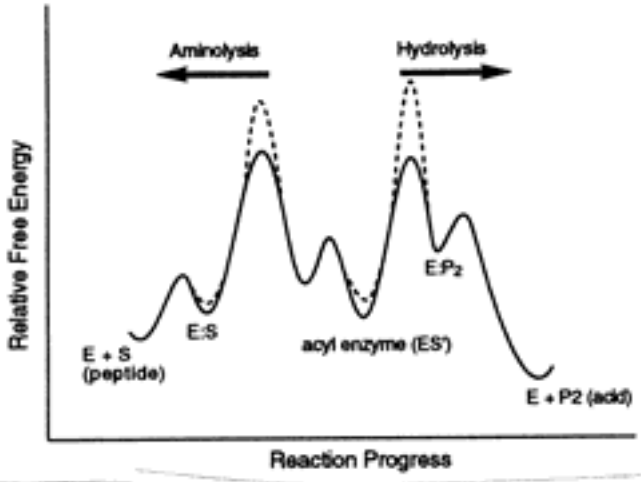


Methylchymotrypsin

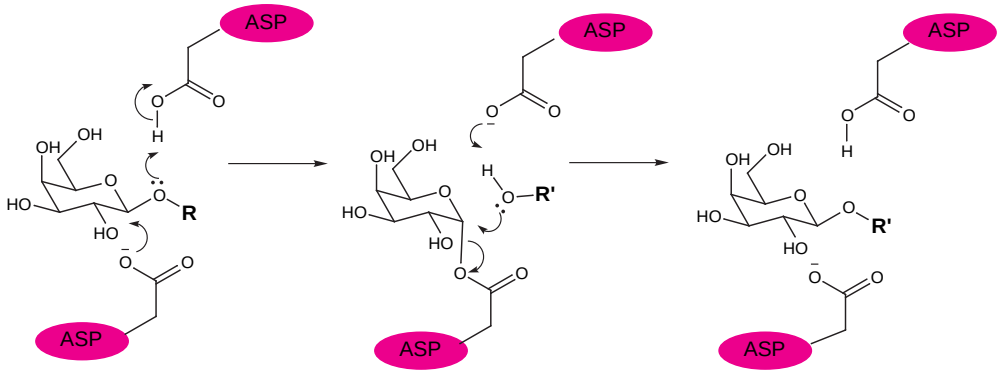


Thiosubtilisin

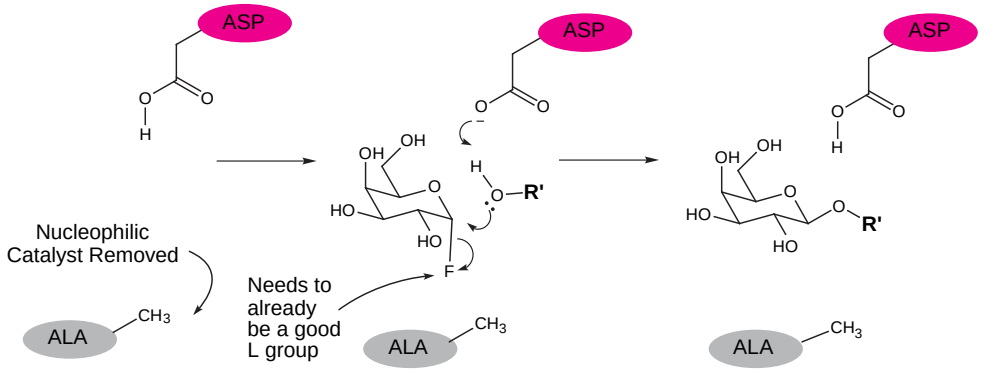




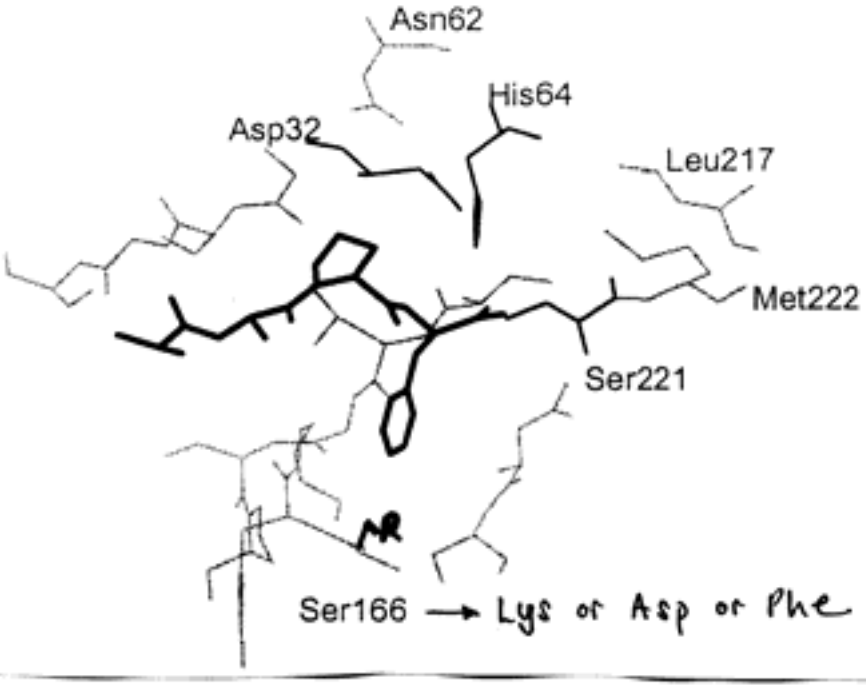
Glycosynthases
Normal β-galactosidase



A "β-galactosynthase"



Engineering Specificity



Amino Acid	Side Chain $-CH_2R$	k_{cat}/K_M* / $s^{-1}mM^{-1}$			
		Phe	Lys	Asp	Ala
Ser	OH	**200**	120	110	30
Lys	$(CH_2)_3NH_3^+$	10	0.2	**240**	40
Asp	COOH	47	**280**	1.2	47
Phe	C_6H_5	5	19	40	**180**

* k_{cat}/K_M is known as the specificity constant as it provides a great idea of the rate of reaction of two competing substrates