

Chem 454 Enzymology Winter 2002
Course Subject Outline

I. ENZYMES AS CATALYSTS (P&S Ch 1 & 2; Fer Ch 2; Palm Ch 1 & 16)

A. Overview--proteins as catalysts (historical background)

B. Enzyme characteristics and properties

C. Enzyme nomenclature/classification

D. Enzyme Purification and Assay

- (1) activity measurements
- (2) enzyme units
- (3) turnover number and properties
- (4) purification and purity
- (5) initial velocity measurements
- (6) assay conditions
- (7) methods for measurement
- (8) choice of assay method
- (9) practical considerations

II. ENZYME KINETICS (P&S Ch 4; Fer Ch 2, 3, 4, 6; Palm Ch 6-8)

A. Kinetics of single substrate reactions

- (1) kinetic concepts
- (2) enzyme kinetics
 - (a) Briggs-Haldane steady-state treatment
 - (b) Michaelis constant (K_m)
 - (c) specificity constant
- (3) graphical analysis

B. Enzyme inhibition

- (1) Classification
 - (a) competitive
 - (b) noncompetitive
 - (c) uncompetitive
 - (d) substrate

C. Multi-substrate reactions

- (1) convention
- (2) mechanisms

D. Substrate binding analysis

- (1) derivation
- (2) methodology

III. MECHANISM OF ENZYME CATALYSIS (P&S Ch 5; Fer Ch 2, 9; Palm Ch 10,11; Zub Ch 9)

A. Reaction Mechanisms and Catalysis

- (1) proximity effect
- (2) acid-base catalysts
- (3) electrostatic
- (4) functional groups
- (5) structural flexibility

B. Active Site Investigations

- (1) kinetic studies
- (2) detection of intermediates
- (3) x-ray crystallographic studies
- (4) chemical modification of amino acid side chains
- (5) site-directed mutagenesis studies

C. Specific enzymes

- (1) alcohol dehydrogenase
- (2) ribonuclease A
- (3) triose phosphate isomerase
- (4) amino acyl tRNA synthetases
- (5) carbonic anhydrase

IV. ENZYME REGULATION (P&S Ch 6; Zub Ch 10)

A. Partial Proteolysis

B. Phosphorylation, adenylation, disulphide reduction

C. Allosteric regulation

- (1) sigmoidal kinetics
- (2) symmetry model
- (3) concerted model
- (4) kinetics and functions of allosteric enzymes
 - (a) phosphofructokinase
 - (b) glycogen phosphorylase

Fer = Ferst; Palm = Palmer, P&S = Price and Stevens; Zub = Zubay.

SUPPLEMENTARY TEXTS

- Alan Fersht (1999) *Structure and Mechanism in Protein Science*, 2nd edition, W.H. Freeman & Co., publ. 1999.
- Nicolas Price & Lewis Stevens (1995) *Fundamentals of Enzymology*, 2nd edition, Oxford Univ. Press, New York, NY., publ 1995.
- Trevor Palmer (1985) *Understanding Enzymes*, Second Edition, J. Wiley & Sons, New York.
- Donald Voet & Judith Voet (1995) *Biochemistry*, J. Wiley & Sons, New York (Chapters 12 through 15).
- Geoffrey Zubay (1993) *Biochemistry*, 3rd edition, Wm. C. Brown, Oxford (1993).
- Robert K. Scopes (1988) *Protein Purification*, Second Edition, Springer-Verlag, Berlin.
- Athel Cornish-Bowden (1979) *Fundamentals of Enzyme Kinetics*, Butterworths, London.
- Methods in Enzymology, Vol. 182 (1990) *Protein Purification*.
- Pharmacia LKB Biotechnology Press: *Gel Filtration, Theory & Practice* and *Affinity Chromatography, Principles & Methods*.
- Laboratory References, a selection of papers cited in the lab manual and collected in a binder for student use.

All indicated supplementary texts, papers and treatises are available at the Reserve Desk at the library on two hour loan.