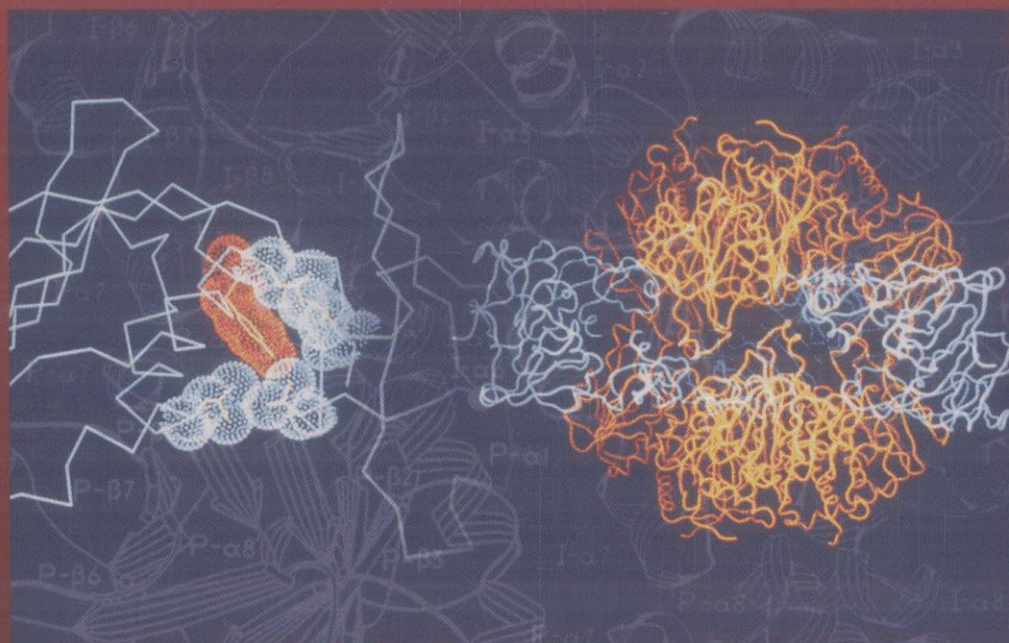




— SELECTED BY GRENOBLE SCIENCES —

Molecular and Cellular Enzymology

Volume I



Jeannine Yon-Kahn - Guy Hervé



Springer

TABLE OF CONTENTS

VOLUME I

GENERAL INTRODUCTION	1
----------------------------	---

PART I – THERMODYNAMICS OF ENZYMES REACTIONS

1 – THE LAWS OF THERMODYNAMICS CONCEPT OF CHEMICAL EQUILIBRIUM.....	15
1.1. <i>The laws of thermodynamics</i>	15
1.1.1. First law.....	15
1.1.2. Second law	16
1.1.3. Third law	18
1.1.4. Free energy.....	19
1.2. <i>Concept of equilibrium – Standard free energy</i>	20
1.3. <i>Experimental determination of thermodynamic parameters</i>	22
1.3.1. Enthalpy change.....	22
1.3.2. Free-energy change	23
1.3.2.1. Thermochemical analysis	24
1.3.2.2. Equilibrium study.....	24
1.3.2.3. Direct measurement of work supplied by the system.....	25
1.4. <i>Coupled reactions</i>	27
1.4.1. Definition of energetic coupling	27
1.4.2. Role of ATP	29
1.4.3. Free energy of hydrolysis of some phosphorylated compounds	30
1.4.4. Some examples of energetic coupling.....	32
1.4.4.1. Formation of ATP from the oxidation energy of nutrients	32
1.4.4.2. Use of the energy from ATP for chemical work.....	33
1.4.4.3. Osmotic work.....	34
1.4.4.4. Mechanical work.....	34
Bibliography	35
2 – PROTEIN-LIGAND ASSOCIATION EQUILIBRIA.....	37
2.1. <i>Proteins possessing a single ligand-binding site</i>	37
2.2. <i>Proteins possessing several equivalent and independent sites</i>	38
2.3. <i>Proteins possessing n independent and non-equivalent sites</i>	41

2.4. <i>Proteins possessing n equivalent but dependent sites</i>	42
2.4.1. Equivalent sites presenting an electrostatic dependence.....	42
2.4.2. Equivalent sites presenting steric or conformational interactions.....	44
2.4.2.1. <i>Phenomenological aspect</i>	44
2.4.2.2. <i>Interaction energy between sites</i>	46
2.4.2.3. <i>Empirical equations</i>	47
2.5. <i>Linked functions</i>	48
2.6. <i>Methods to study ligand binding</i>	51
2.6.1. Equilibrium dialysis.....	51
2.6.2. Dynamic dialysis.....	52
2.6.3. Measuring protein-ligand interactions in a biphasic water-polymer system.....	55
2.6.4. Size-exclusion chromatography.....	55
2.6.5. Ultrafiltration.....	56
2.6.6. Ultracentrifugation.....	56
2.6.7. Direct spectrophotometric methods.....	57
2.6.8. Direct titration of a number of active sites.....	57
2.6.9. Interpretation of experimental data.....	58
<i>Bibliography</i>	61
3 – LIVING BEINGS, OPEN SYSTEMS	63
3.1. <i>Living beings are open systems, far from equilibrium</i>	63
3.1.1. Conservation of mass in open systems.....	64
3.1.2. Energy conservation in open systems: expression of the first law.....	65
3.1.3. Entropy production in open systems: second law.....	66
3.1.4. Entropy production due to chemical reactions: chemical affinity.....	68
3.1.5. Entropy production and rate of irreversible phenomena.....	70
3.1.5.1. <i>Irreversible phenomena in the vicinity of the equilibrium</i>	70
3.1.5.2. <i>Irreversible phenomena far from equilibrium</i>	74
3.2. <i>Exchange of matter and energy with the environment</i>	79
<i>Bibliography</i>	82

PART II – KINETICS OF ENZYMES REACTIONS IN SOLUTION

4 – CHEMICAL KINETICS	85
4.1. <i>Order of chemical reactions</i>	85
4.1.1. Fundamental law of chemical kinetics.....	85
4.1.2. Determining the order of a reaction.....	86
4.1.3. First-order reactions.....	88
4.1.4. Reversible first-order reactions.....	90
4.1.5. Simultaneous first-order reactions.....	92
4.1.6. Second-order reactions.....	93
4.1.6.1. <i>First case: $a_0 \neq b_0$</i>	94
4.1.6.2. <i>Second case: $a_0 = b_0$</i>	94
4.1.7. Reversible second-order reactions.....	94
4.1.8. Dimerisation equilibrium.....	95

4.1.9. Zero-order reactions	96
4.1.10. Significance of the reaction order: order and molecularity	97
4.2. <i>Activation of molecules</i>	97
4.2.1. Activation energy	97
4.2.2. Catalysed reactions – Role of the catalyst	100
5 – KINETICS OF ENZYMATIC REACTIONS WITH MICHAELIAN BEHAVIOUR	103
5.1. <i>Evolution of enzymatic reactions: phenomenological aspects</i>	104
5.1.1. Variation in the quantity of product formed as a function of time	104
5.1.1.1. <i>Pre-steady state phase</i>	104
5.1.1.2. <i>Steady state phase</i>	104
5.1.1.3. <i>Phase of inhibition by the reaction products</i>	104
5.1.1.4. <i>Equilibrium phase</i>	105
5.1.2. MICHAELIS-MENTEN theory	105
5.2. <i>Enzymatic reactions with a single substrate and a single intermediate complex</i>	107
5.2.1. Reversibility of enzymatic reactions	108
5.2.2. Rate of enzymatic reactions: approximation to the steady state, approximation to a quasi-equilibrium Significance of the kinetic parameters	109
5.2.2.1. <i>Kinetics in the pre-steady state</i>	109
5.2.2.2. <i>Reaching the steady state</i>	111
5.2.2.3. <i>Approximation to a quasi-equilibrium</i>	112
5.2.2.4. <i>Order of enzymatic reactions</i>	112
5.2.3. Methods to determine kinetic parameters	116
5.2.3.1. <i>Semi-logarithmic plot</i>	116
5.2.3.2. <i>EADIE plot</i>	116
5.2.3.3. <i>LINEWEAVER-BURK plot</i>	117
5.2.3.4. <i>HANES-DIXON plot</i>	117
5.2.3.5. <i>Plot derived from the integrated rate equation</i>	118
5.2.3.6. <i>Direct plot of EISENTHAL and CORNISHI-BOWDEN</i>	118
5.2.3.7. <i>Validity of the different graphical plots</i>	120
5.3. <i>Kinetics of enzymatic reactions in the presence of effectors (inhibitors or activators)</i>	122
5.3.1. Kinetics of enzymatic reactions in the presence of inhibitors	122
5.3.1.1. <i>Total inhibition</i>	122
5.3.1.2. <i>Partial inhibition</i>	132
5.3.2. Kinetics of enzymatic reactions in the presence of an activator	136
5.3.2.1. <i>Total activation</i>	137
5.3.2.2. <i>Partial activation</i>	139
5.3.2.3. <i>Examples of enzymatic activation</i>	140
5.3.2.4. <i>Activation by the substrate</i>	141
5.4. <i>Enzymatic reactions with one substrate and several intermediate complexes</i>	143
5.4.1. Kinetics at the steady state	143
5.4.1.1. <i>Kinetic analysis by determinants</i>	144
5.4.1.2. <i>Analysis by the graphical method of KING and ALTMAN</i>	144
5.4.1.3. <i>Analysis by other graphical methods</i>	145
5.4.1.4. <i>Relationship between the parameters of the rate equation in reactions with a single substrate and two intermediate complexes</i>	148

5.4.2.	Example: enzymatic reactions catalysed by serine proteases.....	149
5.4.3.	Significance of the kinetic parameters	150
5.4.3.1.	<i>Acylation is limiting: $k_2 \ll k_3$</i>	151
5.4.3.2.	<i>Deacylation is limiting: $k_2 \gg k_3$</i>	151
5.4.4.	Determination of the elementary kinetic constants	151
5.4.5.	Study of nucleophilic competition	153
5.4.5.1.	<i>Study of nucleophilic competition in the case where no binding site exists for water and its analogues</i>	153
5.4.5.2.	<i>Determination of the kinetic parameters in the case where a binding site exists for water and its analogues</i>	158
5.4.6.	Kinetic study of the pre-steady state: titration of enzyme active sites.....	162
5.4.7.	Generalisation for n intermediates	165
5.5.	<i>Enzymatic reactions with two substrates</i>	166
5.5.1.	Nomenclature	166
5.5.2.	Linear schemes	167
5.5.2.1.	<i>Ordered Bi Bi mechanism</i>	167
5.5.2.2.	<i>Iso-ordered Bi Bi mechanism</i>	167
5.5.2.3.	<i>Ping-pong Bi Bi mechanism</i>	168
5.5.2.4.	<i>THEORELL-CHANCE mechanism</i>	168
5.5.3.	Branched schemes: random Bi Bi mechanism	168
5.5.4.	Kinetic study of some two-substrate reactions	168
5.5.4.1.	<i>Ordered Bi Bi mechanism</i>	168
5.5.4.2.	<i>Ping-pong Bi Bi mechanism</i>	172
5.5.4.3.	<i>A branched scheme: the random Bi Bi mechanism</i>	174
5.5.5.	Homeomorphic schemes: how is the ambiguity of the kinetic response removed?.....	177
5.5.5.1.	<i>Studying inhibition by the reaction products: CLELAND's rules</i>	177
5.5.5.2.	<i>Study of substrates binding to an enzyme</i>	179
5.5.5.3.	<i>Study of transitory steps by rapid kinetics</i>	179
5.5.6.	Some examples	179
5.5.6.1.	<i>L-aspartate-2-oxoglutarate amino transferase</i>	179
5.5.6.2.	<i>Yeast hexokinase</i>	181
5.6.	<i>Statistical analysis of experimental data</i>	183
5.6.1.	A few definitions.....	184
5.6.2.	Simple linear regression	184
5.6.3.	Multilinear regression	186
5.6.4.	Non-linear regression analysis	186
5.6.5.	Checking the adequacy of the fit.....	187
5.6.5.1.	<i>Examination of the residual values</i>	187
5.6.5.2.	<i>The weighting factor, w_i</i>	188
5.6.5.3.	<i>General strategy</i>	189
	<i>Bibliography</i>	189
6 –	EXPERIMENTAL METHODS TO STUDY ENZYMATI C REACTIONS.....	193
6.1.	<i>Discontinuous methods</i>	194
6.2.	<i>Continuous methods</i>	194
6.3.	<i>Coupled enzyme assays</i>	196
6.4.	<i>Flow methods</i>	202

6.4.1. General principle of flow methods.....	202
6.4.2. Continuous-flow apparatus	202
6.4.3. Stopped-flow apparatus.....	204
6.4.4. Quenched-flow apparatus.....	205
6.4.5. Criterion for homogeneity of the mixture	205
6.4.6. Some technical problems	206
6.5. <i>Relaxation methods</i>	206
6.5.1. Principle of relaxation methods.....	207
6.5.1.1. <i>Transient perturbation</i>	207
6.5.1.2. <i>Alternative perturbation</i>	208
6.5.2. Principal relaxation methods.....	209
6.5.2.1. <i>Thermal relaxation</i>	209
6.5.2.2. <i>Pressure shock</i>	210
6.5.2.3. <i>Other relaxation methods</i>	210
6.5.3. Analysis of kinetic data.....	210
6.5.3.1. <i>Reactions with n consecutive steps</i>	211
6.5.3.2. <i>First-order reaction with a single step: isomerisation</i>	212
6.5.3.3. <i>Bimolecular reaction with one step</i>	213
6.5.3.4. <i>Bimolecular reaction followed by isomerisation</i>	214
6.5.3.5. <i>Dimerisation equilibrium</i>	216
6.5.3.6. <i>Analysis of relaxation data</i>	217
6.5.3.7. <i>Example of studying an enzymatic reaction by means of thermal relaxation</i>	218
6.6. <i>Study of enzymatic reactions at low temperatures: cryoenzymology</i>	220
6.7. <i>Study of enzymatic reactions under high pressure</i>	221
6.7.1. Principle	221
6.7.2. Activation volume.....	222
6.7.3. Equipment.....	223
<i>Bibliography</i>	225

PART III – FORMATION AND STRUCTURE OF THE ACTIVE CENTRE OF ENZYMES

7 – ENZYME ORIGIN AND EVOLUTION	229
7.1. <i>Time-scale of evolution</i>	229
7.2. <i>Prebiotic chemistry according to the “original soup” hypothesis</i>	231
7.2.1. Formation of some simple organic molecules.....	231
7.2.2. Formation of macromolecules.....	232
7.2.2.1. <i>Abiotic formation of polypeptides</i>	233
7.2.2.2. <i>Abiotic formation of nucleotides and nucleic acids</i>	233
7.2.3. Discussion of the nature of the first biological molecules.....	234
7.3. <i>Theory of surface metabolism</i>	236
7.3.1. Autotrophic surface “metabolists”	238
7.3.2. The change towards cellular metabolism	240
7.3.3. Evolution of the genetic apparatus	242
7.3.4. Catalytic properties of RNA.....	245

7.4. Chirality of biological molecules	247
7.5. Other theories on the origin of life	248
Bibliography	249
8 – FORMATION OF THE FUNCTIONAL STRUCTURE OF ENZYMES:	
CO- AND POST-TRANSLATIONAL EVENTS	251
8.1. Covalent processes	251
8.1.1. Limited proteolysis.....	251
8.1.2. Chemical modifications	257
8.2. Non-covalent processes	261
8.2.1. Protein folding.....	261
8.2.2. Assembly of subunits	264
Bibliography	264
9 – TOPOLOGY OF THE ACTIVE CENTRE OF ENZYMES	267
9.1. Kinetic approach – Analysis of pH profiles	269
9.1.1. Effect of pH on the conformational state of the protein	270
9.1.1.1. Irreversible denaturation	270
9.1.1.2. Reversible denaturation	270
9.1.1.3. Ionisation of groups that interfere specifically with the active conformation of the enzyme	270
9.1.2. Substrate ionisation states	271
9.1.3. Effect of pH on enzyme-substrate associations.....	272
9.1.4. Effects of pH on the ionisation of catalytic groups	274
9.1.4.1. Enzymatic reactions involving a single intermediate	274
9.1.4.2. Enzymatic reactions implicating two intermediate complexes.....	278
9.1.4.3. Enzymatic reactions involving several intermediate complexes	279
9.2. Chemical approach to studying the active centre of enzymes.....	280
9.2.1. Principle of chemical labelling.....	280
9.2.1.1. Effects of the microenvironment on the protein functional groups.....	281
9.2.1.2. Effect of microenvironment on the reagent	284
9.2.1.3. Required conditions for conducting a chemical modification	285
9.2.2. Strategy of chemical modifications	286
9.2.2.1. Labelling by a substrate, a quasi-substrate or a coenzyme	286
9.2.2.2. Affinity labelling	290
9.2.2.3. Photoaffinity labelling	295
9.2.2.4. Suicide reagents	299
9.2.2.5. Direct labelling by selective reagents	300
9.2.2.6. Differential labelling	301
9.2.2.7. Principal reactions of amino acid side chains	302
9.2.3. Criteria used to interpret results	322
9.2.3.1. Stoichiometric inactivation.....	322
9.2.3.2. Specific protection against inactivation	322
9.2.3.3. Kinetic analysis of results.....	323
9.2.3.4. Reversibility of the chemical modification and the loss of activity.....	331

9.3. Use of mutagenesis methods to study the active centre of enzymes	331
9.3.1. Methodology	332
9.3.2. Strategy	333
9.3.3. Some examples	334
9.4. Structural studies by radiocrystallography and nuclear magnetic resonance of the active centre of enzymes	334
Bibliography	337

VOLUME II

PART IV – THE CATALYTIC FUNCTION

INTRODUCTION	343
10 – FORMATION OF ENZYME-SUBSTRATE COMPLEXES	347
10.1. Nature of forces involved in enzyme-substrate associations	348
10.1.1. Electrostatic interaction forces	348
10.1.1.1. Interactions between two ions or coulomb interactions	348
10.1.1.2. Interactions between an ion and a dipole	350
10.1.1.3. Interactions between permanent dipoles	351
10.1.2. Induction interactions.....	353
10.1.2.1. Interaction between an ion and an induced dipole	353
10.1.2.2. Interactions between a dipole and an induced dipole or DEBYE interactions	354
10.1.3. Electrokinetic interactions or LONDON dispersion forces	355
10.1.4. Short-range repulsive interactions.....	356
10.1.5. The hydrogen bond	358
10.1.6. Hydrophobic interactions	359
10.1.7. The covalent bond.....	360
10.1.8. Determination of the nature of enzyme-substrate interactions.....	360
10.2. Energetics of enzyme-substrate associations.....	365
10.3. Mechanisms of enzyme-substrate association	370
10.3.1. Induced fit theory	370
10.3.2. “Rack” or “strain” theory (distortions or constraints in the substrate).....	373
10.3.3. “Dynamic rack” theory	373
Bibliography	374
11 – CATALYTIC MECHANISMS	377
11.1. Chemical catalysis.....	378
11.1.1. Definitions and general principles.....	378
11.1.1.1. Mechanisms of breaking a covalent bond.....	378
11.1.1.2. Nucleophilic and electrophilic reactions.....	379
11.1.1.3. Transition state theory.....	380
11.1.2. Nucleophilic catalysis	380
11.1.2.1. Formation of the addition intermediate: the tetrahedral complex	381

11.1.2.2. <i>Effect of structure on reactivity</i>	382
11.1.2.3. <i>Possible mechanisms of nucleophilic catalysis</i>	384
11.1.3. <i>General base catalysis</i>	385
11.1.4. <i>Electrophilic catalysis</i>	387
11.1.5. <i>General acid catalysis</i>	389
11.1.6. <i>General acid-base catalysis</i>	391
11.2. <i>Isotope effects</i>	393
11.2.1. <i>Primary isotope effects</i>	393
11.2.1.1. <i>Definition</i>	393
11.2.1.2. <i>Energy of primary isotope effects</i>	394
11.2.1.3. <i>Isotope effects with tritium</i>	395
11.2.2. <i>Solvent effects: equilibria in H₂O and D₂O</i>	395
11.2.3. <i>Secondary isotope effects</i>	397
11.2.3.1. <i>Change in frequency of bonds between non-reacting atoms</i>	398
11.2.3.2. <i>Induced effects</i>	398
11.2.3.3. <i>Hyperconjugation</i>	398
11.2.3.4. <i>Steric effects</i>	398
11.2.3.5. <i>Solvent effects</i>	399
11.2.4. <i>Magnitude of isotope effects</i>	399
11.2.5. <i>Isotope effects on enzyme reactions</i>	400
11.3. <i>Principal types of reactions catalysed by enzymes</i>	401
11.3.1. <i>Group transfer reactions</i>	401
11.3.1.1. <i>Acyl transfer reactions</i>	402
11.3.1.2. <i>Phosphoryl group transfer</i>	402
11.3.1.3. <i>Glycosyl group transfer</i>	404
11.3.2. <i>Oxydoreduction reactions</i>	404
11.3.3. <i>Elimination, isomerisation and rearrangement reactions</i>	408
11.3.4. <i>Formation or breaking of carbon-carbon bonds</i>	410
11.4. <i>Particularities of enzyme catalysis</i>	412
11.4.1. <i>Enzyme catalysis is an intramolecular catalysis</i>	413
11.4.1.1. <i>Enzyme reactions are first order reactions</i>	413
11.4.1.2. <i>Concentration effect</i>	414
11.4.1.3. <i>Orientation effects</i>	416
11.4.1.4. <i>Entropy effects</i>	421
11.4.1.5. <i>Role of induced fit and constraints</i>	422
11.4.2. <i>Enzyme catalysis is a polyfunctional catalysis</i>	423
11.4.3. <i>Complementarity of the enzyme for the transition state of the substrate</i>	425
11.4.3.1. <i>Energy aspects</i>	425
11.4.3.2. <i>Kinetic parameters corresponding to several enzyme reactions</i>	427
11.4.3.3. <i>Affinity of enzymes for transition state analogs</i>	431
11.4.3.4. <i>Estimation of minimal affinities of enzymes for transition states of substrates</i>	433
11.4.3.5. <i>Structural arguments</i>	434
11.4.3.6. <i>One application of this particularity: abzymes</i>	439
11.4.4. <i>Effects of microenvironment</i>	441
11.4.4.1. <i>Electrostatic effects</i>	441
11.4.4.2. <i>Role of water molecules</i>	444
11.4.4.3. <i>Role of the hydrophobic environment</i>	445
11.4.4.4. <i>Low barrier hydrogen bonds</i>	445

11.4.5. Reaction intermediates in enzyme catalysis.....	446
11.5. Conclusions.....	448
Bibliography.....	448
12 – EXAMPLES OF STRUCTURE-FUNCTION RELATIONSHIPS IN ENZYMATIC SYSTEMS	451
12.1. Proteases.....	452
12.1.1. Serine proteases.....	452
12.1.1.1. Structural aspects.....	452
12.1.1.2. Activation of zymogens.....	453
12.1.1.3. The reaction pathway.....	458
12.1.1.4. The binding site of substrates and the MICHAELIS complex.....	459
12.1.1.5. The tetrahedral complex and the binding site of the oxonium ion.....	461
12.1.1.6. The catalytic triad and the mechanism of acylation.....	462
12.1.1.7. The acyl-enzyme and the deacylation step.....	463
12.1.2. Thiol proteases.....	465
12.1.2.1. Structural aspects.....	466
12.1.2.2. Activation of thiol proteases.....	468
12.1.2.3. The active centre.....	469
12.1.2.4. Catalytic mechanism.....	471
12.1.3. Acid proteases or aspartyl proteases.....	471
12.1.3.1. Activation of zymogens.....	472
12.1.3.2. Structural aspects.....	473
12.1.3.3. Enzyme-substrate association.....	474
12.1.3.4. The catalytic site.....	476
12.1.3.5. Formation of the tetrahedral intermediate.....	477
12.1.3.6. Breaking of the tetrahedral intermediate.....	477
12.1.4. Metalloproteases: carboxypeptidase A.....	478
12.1.4.1. Zymogen activation.....	480
12.1.4.2. Structure of carboxypeptidase A.....	481
12.1.4.3. Localisation and role of Zn^{2+}	482
12.1.4.4. The active centre.....	484
12.1.4.5. Enzyme-substrate association.....	485
12.1.4.6. Catalytic mechanisms.....	486
12.2. Phosphoryl transfer enzymes.....	489
12.2.1. Amino-acyl tRNA synthetases.....	489
12.2.2. Kinases - Phosphoglycerate kinase.....	490
12.2.2.1. Structural properties.....	491
12.2.2.2. The binding site of nucleotide substrates.....	491
12.2.2.3. The binding site of phosphoglycerate substrates.....	493
12.2.2.4. The ternary complex and the movement of domains.....	494
12.2.2.5. Catalytic mechanism.....	495
12.3. Glycosyl transfer enzymes - Lysozyme.....	496
12.3.1. Structural properties.....	497
12.3.2. The active centre.....	498
12.3.3. Catalytic mechanism.....	499
12.4. Oxydoreduction enzymes.....	501
12.4.1. Alcohol dehydrogenase.....	501
12.4.1.1. Structural properties.....	501

12.4.1.2. Conformational change of the enzyme induced by the coenzyme binding.....	503
12.4.1.3. Binding of the coenzyme	505
12.4.1.4. Binding of substrates	506
12.4.1.5. Catalytic mechanism	506
12.4.2. Flavocytochrome b_2	507
12.4.2.1. Structural properties	507
12.4.2.2. Binding of heme and flavine	509
12.4.2.3. Binding of the substrate	510
12.4.2.4. Reaction mechanism.....	512
12.5. Triose phosphate isomerase	513
12.5.1. Structure of the enzyme	513
12.5.2. Structure of the enzyme-substrate complex	514
12.5.3. Reaction mechanism	514
12.6. Aspartate aminotransferase.....	518
12.6.1. Structural properties.....	520
12.6.2. Binding of the coenzyme	522
12.6.3. Binding of the substrate: conformational change of the enzyme	523
12.6.4. The active site	524
12.6.5. Catalytic mechanism.....	524
12.7. Aldolases	526
12.7.1. Structural properties.....	528
12.7.2. The active site	530
12.7.3. Catalytic mechanism.....	532
12.8. Conclusions and perspectives.....	534
Bibliography.....	537

PART V – REGULATION OF ENZYME ACTIVITY

INTRODUCTION.....	545
13 – REGULATION BY NON-COVALENT INTERACTIONS.....	547
13.1. Allosteric regulation.....	547
13.2. Phenomenological aspect of cooperativity.....	548
13.3. Phenomenological models.....	550
13.3.1. The HILL equation.....	550
13.3.2. The ADAIR equation.....	552
13.4. The concerted model [MONOD, WYMAN & CHANGEUX, 1965].....	553
13.4.1. Definition	553
13.4.2. K systems	554
13.4.3. V systems in the concerted model.....	559
13.5. Sequential model [KOSHLAND, NÉMÉTHY & FILMER, 1966].....	561
13.5.1. Definition	561
13.5.2. Tetrahedral tetramer model.....	563
13.5.3. Square tetramer model	564
13.5.4. Anticooperativity	564

13.6. <i>The generalised model</i>	567
13.7. <i>Thermodynamical coupling between ligand binding energy and subunit interaction energy</i>	567
13.8. <i>Kinetic cooperativity: RICHARD model</i>	570
13.9. <i>Cooperativity and allostery</i>	572
13.10. <i>Examples of allosteric enzymes</i>	572
13.10.1. <i>Glycogen phosphorylase</i>	573
13.10.1.1. <i>Allosteric regulation</i>	573
13.10.1.2. <i>Phosphorylase structure</i>	576
13.10.2. <i>Phosphofructokinase</i>	579
13.10.2.1. <i>Structure of phosphofructokinase</i>	580
13.10.2.2. <i>Phosphofructokinase allosteric regulation</i>	581
13.10.3. <i>E. coli aspartate transcarbamylase</i>	585
13.10.3.1. <i>Cooperative effects between catalytic sites</i>	587
13.10.3.2. <i>Allostery - Heterotropic interactions between regulatory and catalytic sites</i>	592
13.10.4. <i>Ribonucleotide reductase</i>	596
13.10.4.1. <i>Reaction mechanism</i>	596
13.10.4.2. <i>Ribonucleotide reductase structure</i>	597
13.10.4.3. <i>Allosteric regulation</i>	599
13.11. <i>"Squatting"</i>	601
13.12. <i>"Mnemonic" enzymes</i>	602
13.12.1. <i>Case of a mnemonic enzyme with one substrate and one product</i>	605
13.12.1.1. <i>Kinetic behaviour</i>	605
13.12.1.2. <i>Thermodynamic aspects</i>	606
13.12.2. <i>Case of a mnemonic enzyme with two substrates and two products</i>	607
13.12.3. <i>The reaction product acts as an effector</i>	610
13.13. <i>Regulation through protein-protein interaction</i>	612
13.13.1. <i>The lipase-colipase system</i>	613
13.13.2. <i>Regulation of ornithine transcarbamylase from <i>Saccharomyces cerevisiae</i> by arginase</i>	618
13.13.3. <i>cAMP dependent protein kinases</i>	620
13.13.4. <i>Regulations by interaction with the calmodulin-calcium complex</i>	623
<i>Bibliography</i>	626
14 – REGULATION BY COVALENT MODIFICATION	631
14.1. <i>Modifications by limited proteolysis: activation of precursors</i>	631
14.2. <i>Protein inhibitors of proteases</i>	632
14.2.1. <i>Serine protease inhibitors</i>	632
14.2.1.1. <i>α_2-macroglobulins</i>	632
14.2.1.2. <i>Protein protease inhibitors that possess a specific cleavage site</i>	633
14.2.2. <i>Thiol protease inhibitors</i>	639
14.2.3. <i>Protein inhibitors of metalloproteases</i>	641
14.2.4. <i>Aspartyl protease protein inhibitors</i>	641
14.3. <i>Regulation via chemical modification</i>	641
14.3.1. <i>Phosphorylation</i>	641

14.3.2.	ADP-ribosylation.....	644
14.3.2.1.	Enzymatic ADP ribosylations.....	644
14.3.2.2.	Physiological effects.....	646
14.3.3.	Glycosylation.....	647
14.3.4.	Adenylation. uridylation.....	649
14.4.	Cascade systems - Mechanism of action.....	653
14.4.1.	Definition.....	653
14.4.2.	Irreversible cascade systems.....	654
14.4.2.1.	Blood clotting cascade.....	654
14.4.2.2.	The complement system.....	659
14.4.3.	Cyclic cascades.....	661
14.4.3.1.	Monocyclic cascades.....	661
14.4.3.2.	Bicyclic cascade systems.....	666
14.4.3.3.	Multicyclic cascade systems.....	669
14.5.	Irreversible inactivations.....	670
14.5.1.	Proteasomes.....	670
14.5.1.1.	20s proteasome.....	671
14.5.1.2.	26s proteasome.....	672
14.5.2.	Caspases and apoptosis.....	673
	Bibliography.....	677

15 – MULTIFUNCTIONAL ENZYMES, MULTI-ENZYMATIC COMPLEXES

	AND METABOLIC CHANNELLING.....	679
15.1.	Phosphoribosylanthranilate isomerase-indole glycerolphosphate synthase.....	680
15.1.1.	Structure of the <i>E. coli</i> enzyme.....	681
15.1.2.	Structure of the active site.....	683
15.1.3.	Functional properties.....	685
15.2.	Tryptophan synthase.....	687
15.2.1.	Functional properties.....	687
15.2.2.	Enzyme structure.....	691
15.2.3.	Study of a mutant leading to channel obstruction.....	694
15.3.	<i>Cad</i> protein.....	695
15.3.1.	The first enzymes in the pyrimidine biosynthesis pathway.....	695
15.3.2.	Structural aspects.....	696
15.3.3.	Functional properties.....	697
15.4.	Carbamyl phosphate synthetase.....	697
15.4.1.	Functional properties.....	697
15.4.2.	Structural properties.....	698
15.4.3.	The tunnel.....	699
15.5.	The pyruvate dehydrogenase complex.....	700
15.5.1.	Functional properties.....	700
15.5.2.	Structural properties.....	702
15.5.3.	Role of lipoamide domains in substrate channelling.....	705
15.6.	Fatty acid synthetase.....	706
15.6.1.	Functional properties.....	706

15.6.2. Structural characteristics.....	709
15.7. <i>Transitory multienzymatic complexes and channelling</i>	712
<i>Bibliography</i>	720

PART VI – ENZYMOLOGY IN STRUCTURED ENVIRONMENT

INTRODUCTION	725
16 – LOCALISATION AND CELLULAR COMPARTMENTALISATION	727
16.1. <i>Localisation of enzymes in cellular compartments</i>	727
16.2. <i>The cellular concentrations of macromolecules</i>	731
16.3. <i>Interactions of enzymes with cellular constituents</i>	733
16.3.1. Membrane enzymes.....	733
16.3.2. Enzymes associated to the cytoskeleton.....	735
16.3.3. Enzymes associated to plant cell walls.....	736
16.4. <i>Compartmentalisation of metabolites</i>	737
17 – KINETICS OF ENZYMATIC REACTIONS CATALYSED BY IMMOBILISED ENZYMES	741
17.1. <i>Fundamental coupling equation</i>	741
17.2. <i>Hysteresis loops in reactions involving diffusion-reaction coupling</i>	743
17.3. <i>Electrostatic constraints on immobilised enzymes</i>	745
18 – METABOLIC CONTROL THEORY	749
18.1. <i>Control of a linear metabolic pathway at steady state</i>	749
18.1.1. Control coefficients.....	750
18.1.2. Elasticity coefficients and the connectivity relation.....	751
18.1.3. Experimental approaches.....	754
18.2. <i>Control of metabolic cycles</i>	755
<i>Bibliography</i>	761
CONCLUSIONS AND PERSPECTIVES	763
<i>Bibliography</i>	766
GENERAL BIBLIOGRAPHY	767
PHYSICAL CONSTANTS	769
INDEX	771