

Contents

Part I Proteins

1	Amino Acids	3
1.1	Basic Structure of Amino Acids.....	3
1.2	The Isoelectric Point.....	4
1.3	The One-Letter Code.....	9
1.4	Biological Function of Amino Acid Variety.....	10
1.5	Exercises.....	12
	1.5.1 Problems.....	12
	1.5.2 Solutions.....	12
	Reference.....	13
2	Protein Structure	15
2.1	Primary Structure.....	15
	2.1.1 Protein Sequences and Evolution.....	18
2.2	Secondary Structure.....	20
	2.2.1 The α -Helix.....	22
	2.2.2 β -Strand.....	24
	2.2.3 The P _{II} (syn.: Poly-Pro or Polypeptide II) Helix.....	28
	2.2.4 Hairpin Turns.....	30
	2.2.5 Rare Structures.....	31
	2.2.6 Coils.....	32
2.3	Tertiary Structure.....	32
	2.3.1 Classification of Proteins by Folding Pattern.....	34
2.4	Quaternary Structure.....	37
2.5	Further Aspects of Protein Structure.....	38
	2.5.1 LEVINTHAL's paradox:.....	38
	2.5.2 Energetics and Kinetics of Protein Folding.....	40
	2.5.3 Morpheins.....	41
	2.5.4 Molecular Chaperones and Chaperonins.....	42
	2.5.5 Protein Denaturation.....	42
	2.5.6 Protein Folding.....	43

2.6	Posttranslational Modifications of Proteins	47
2.6.1	Glycosylation	47
2.6.2	Glucation	49
2.6.3	Disulphide Bond Formation.....	50
2.6.4	Proteolysis	51
2.6.5	Hydroxylation.....	52
2.6.6	Phosphorylation/Dephosphorylation	53
2.6.7	Acetylation/Deacetylation.....	53
2.6.8	Methylation/Demethylation	54
2.6.9	Addition/Removal of Hydrophobic Tails	55
2.6.10	S-Nitrosylation.....	55
2.6.11	ADP-Ribosylation	55
2.6.12	Deamidation	55
2.6.13	AMPylation (Adenylylation).....	57
2.6.14	Transfer of Peptides	57
2.7	The Relationship Between Protein Structure and Function: Green Fluorescent Protein	59
2.8	Exercises	61
2.8.1	Problems	61
2.8.2	Solutions	62
	References.....	62
3	Proteins in the Lab	65
3.1	Protein Purification	65
3.1.1	Homogenisation and Fractionisation of Cells and Tissues.....	65
3.1.2	Precipitation Methods	66
3.1.3	Chromatography	67
3.1.4	Electrophoresis	71
3.1.5	Membrane Proteins	73
3.2	Determination of Protein Concentration	76
3.3	Protein Sequencing	78
3.3.1	Edman Degradation	78
3.3.2	Mass Spectrometry	79
3.3.3	Phylogenetic Trees	83
3.4	Synthesis of Peptides.....	85
3.5	How Do We Determine Secondary Structure?.....	85
3.5.1	X-Ray Crystallography	86
3.5.2	Electron Microscopy	87
3.5.3	Nuclear Magnetic Resonance	87
3.5.4	Computer Predictions	88
3.6	Exercises.....	89
3.6.1	Problems	89
3.6.2	Solutions	91
	References.....	91

Part II Enzymes

4 Enzymes Are Biocatalysts	97
4.1 The Nature of Catalysis	97
4.1.1 A Brief History of Enzymology	98
4.2 Enzyme Classification and EC Code	101
4.2.1 Chemical and Biological Direction of a Reaction.....	104
4.3 Inherited Diseases of Metabolism	104
4.4 Exercises	109
4.4.1 Problems	109
4.4.2 Solutions	109
References	110
5 Enzyme Kinetics and Mechanism	111
5.1 The HENRI–MICHAELIS–MENTEN Equation	111
5.1.1 Efficiency Constant and Catalytic Perfection	117
5.1.2 Application: Forensic Determination of Blood Alcohol Concentration	119
5.1.3 Linearisation of the HMM-Equation	120
5.1.4 Experimental Pitfalls	123
5.1.5 Environmental Influences on Enzyme Activity	126
5.2 Enzymes with Several Substrates.....	126
5.2.1 Nomenclature	127
5.2.2 How Do We Determine the Mechanism of Multisubstrate Enzymes?.....	128
5.3 Enzyme Precursors and Their Activation	129
5.4 The Coupled Spectrophotometric Assay of WARBURG	130
5.5 How Do Enzymes Work?	131
5.5.1 Molecular Mechanism of Serine-Proteases and -Esterases	133
5.6 Exercises	135
5.6.1 Problems	135
5.6.2 Solutions	137
References	139
6 Inhibition and Inactivation of Enzymes	141
6.1 Enzyme Inhibition	141
6.2 Competitive Inhibition	142
6.3 Uncompetitive Inhibition	147
6.4 Noncompetitive Inhibition	148
6.5 Partial Inhibition.....	151
6.6 Inactivation of Enzymes	154
6.7 Exercises	157
6.7.1 Problems	157
6.7.2 Solutions	160
References	161

7	Hæmoglobin and Myoglobin: Cooperativity	163
7.1	Structure	165
7.2	Oxygen Binding and Cooperativity	165
7.2.1	Functional Significance of Cooperativity.....	167
7.2.2	Mechanism of Cooperativity	168
7.2.3	Other Factors Involving Oxygen Affinity of Hæmoglobin	173
7.3	Hæmoglobin Related Diseases	176
7.3.1	Subunit Composition of Human Hæmoglobin	176
7.3.2	Inherited Diseases Relating to Hæmoglobin	177
7.4	Exercises	183
7.4.1	Problems	183
7.4.2	Solutions	183
	References.....	183
8	Enzyme Kinetics: Special Cases	185
8.1	Activation Cascades	185
8.2	Feedback-Networks	188
8.3	Multiple Phosphorylation	190
	References.....	191
9	The Flow of Metabolites Through Metabolic Pathways	193
9.1	Flux Control Theory	194
9.1.1	Supply/Demand Analysis	197
9.1.2	Mechanism of Flux Control	197
	References.....	199
Part III Special Proteins		
10	Protein Folding Diseases	203
10.1	Intrinsically Disordered Proteins	203
10.1.1	Protein-Only Elements of Inheritance	205
10.2	Amyloidoses	206
10.2.1	Prion Proteins and Prion Diseases	207
10.2.2	Neuronal Amyloidoses	213
10.2.3	Amyloidoses in Other Organs.....	218
10.2.4	Amyloidoses Secondary to Other Diseases	220
10.3	Exercises.....	221
10.3.1	Problems	221
10.3.2	Solutions	221
	References.....	222
11	Immunoproteins	225
11.1	Overview.....	226
11.1.1	Cells of the Immune System	227
11.2	Humoral Immunity: Immunoglobulins	231
11.2.1	Structure of Immunoglobulins	231

11.2.2	How Is the Large Number of Ig-Molecules Obtained? ...	234
11.2.3	Time Course of Antibody Response	241
11.2.4	Immunisation	244
11.2.5	Monoclonal Antibodies	246
11.2.6	Laboratory Uses of Antibodies	247
11.3	Destroying Invaders: The Complement System	249
11.3.1	How Is Complement Activated?	251
11.3.2	What Does Complement Do?	253
11.3.3	How Is Complement Inactivated?.....	256
11.4	Cellular Immunity	258
11.4.1	The Major Histocompatibility Complex.....	258
11.4.2	The T-Cell Receptor	267
11.5	Proteins Involved in Innate Immunity	270
11.5.1	PAMP-Receptors	270
11.5.2	Cytokines	275
11.5.3	The Acute-Phase Response	277
11.5.4	Antibacterial Proteins	278
11.6	Exercises	281
11.6.1	Problems	281
11.6.2	Solutions	282
	References.....	283
12	Cell Skeleton	287
12.1	The Microfilament.....	288
12.1.1	Basic Actin Structure	288
12.1.2	Actin-Binding Proteins	291
12.1.3	Functions of Actin	295
12.1.4	Actin-Networks	295
12.2	Microtubules	296
12.2.1	Microtubule Structure	297
12.3	Intermediate Filament	299
12.3.1	IF-Proteins Are Cell-Type Specific	300
12.3.2	Structure of Intermediate Filaments	302
12.3.3	Intermediate Filaments and Cell Cycle	303
12.3.4	Other Proteins Associated with Intermediate Filaments.....	303
12.4	Exercises	304
12.4.1	Problems	304
12.4.2	Solutions	304
	References.....	304
13	Motor Proteins and Movement.....	305
13.1	Myosin Moves Along Actin Filaments.....	305
13.1.1	Myosin Structure	305
13.1.2	Myosin-II	306

13.1.3	Myosin-I	310
13.1.4	Myosin-V	311
13.2	Kinesin and Dynein Move Along Microtubules	311
13.2.1	Kinesin Is Responsible for Anterograde (Minus to Plus) Transport	311
13.2.2	Dynein Is Responsible for Retrograde (Plus to Minus) Movement	313
13.3	Cilia and Flagella.....	313
13.3.1	Generic Structure of Cilia and Flagella.....	313
13.3.2	Mechanism of Movement	315
13.3.3	Cilia and Flagella Start Growing at the Basal Body	317
13.4	The Mitotic Spindle	317
	References	322
14	Cell–Cell Interactions	323
14.1	Extracellular Matrix	323
14.1.1	Collagen.....	324
14.1.2	Elastin	331
14.2	Cell Adhesion Molecules	335
14.3	Junctions	337
14.3.1	Disintegrins	339
14.4	Exercises	339
14.4.1	Problems	339
14.4.2	Solutions	340
	References.....	340
15	Aiding in Folding: Molecular Chaperones and Chaperonins.....	343
15.1	Hsp70 Is an Example for Molecular Chaperones	345
15.2	Other Heat Shock Proteins also Have Chaperone Activity.....	350
15.2.1	Hsp90	350
15.2.2	Small Heat Shock Proteins	353
15.3	The GroEL/GroES-Foldosome Is an Example for Molecular Chaperonins	354
15.4	Exercises.....	357
15.4.1	Problems	357
15.4.2	Solutions	358
	References.....	358
Part IV Membrane Transport		
16	Protein Transport Across Membranes	363
16.1	Structure of Membrane Components	363
16.1.1	Membrane Lipid	363
16.1.2	Membrane Proteins	364
16.2	Transport of Proteins into Mitochondria	368
16.2.1	The Mitochondrion in the Cell Cycle.....	368
16.2.2	Mitochondrial Proteins and Lipids.....	369

16.3	Synthesis and Sorting of Mitochondrial Proteins in the Cytosol...	371
16.4	Transfer of Protein into the ER Lumen.....	375
16.5	Folding and Quality Control of Membrane Proteins	377
16.5.1	Posttranslational Modification in the ER	377
16.5.2	Glycosylation	379
16.5.3	Protein Quality Control in the ER	385
16.6	Exercises.....	387
16.6.1	Problems	387
16.6.2	Solutions	389
	References.....	389
17	Vesicular Transport in Eukaryotic Cells	393
17.1	Two Models for Transport Between Compartments	393
17.1.1	Maturation	393
17.1.2	Vesicular Transport	395
17.1.3	Experimental Evidence	395
17.2	Clathrin	395
17.2.1	Endocytosis, Membrane Protein Recycling, and Transcytosis	396
17.3	β -Coat Protein	403
17.3.1	Vesicular Transport Between ER and GOLGI Stacks	403
17.4	The Specificity of Membrane Fusion.....	409
17.4.1	v-SNAREs and t-SNAREs	409
17.4.2	Rab Proteins	409
17.4.3	How Do Proteins Know Where They Belong?.....	410
17.5	Other Vesicular Transport Pathways	412
17.5.1	Transport of Newly Synthesised Proteins to Their Destination	412
17.5.2	Proteins Taken Up by the Cell	416
17.6	Exercises.....	417
17.6.1	Problems	417
17.6.2	Solutions	418
	References.....	419
18	Transport of Solutes Across Membranes	421
18.1	Passive Diffusion	422
18.2	Transporters	423
18.2.1	Primary Active Transporters (Pumps)	424
18.2.2	Secondary Active Transporter (Cotransporter).....	454
18.2.3	Facilitated Diffusion.....	458
18.3	Exercises.....	465
18.3.1	Problems	465
18.3.2	Solutions	466
	References.....	467

Appendix A: Short Biographies of Scientists Mentioned in This Book	469
Appendix B: List of Symbols.....	489
Appendix C: Greek Alphabet.....	491
Appendix D: The Genetic Code	493
Appendix E: Acronyms	495
Index.....	507