

Contents

Preface

xi

1 Chemical Properties of Polypeptides

1

1.1 The Polymeric Nature of Proteins

2

1.2 The Polypeptide Backbone

5

1.3 Amino Acid Residues

6

1.3.1 Gly

7

1.3.2 The Aliphatic Residues: Ala, Val, Leu, Ile

7

1.3.3 The Cyclic Imino Acid: Pro

7

1.3.4 The Hydroxyl Residues: Ser and Thr

8

1.3.5 The Acidic Residues: Asp and Glu

8

1.3.6 The Amide Residues: Asn and Gln

9

1.3.7 The Basic Residues: Lys and Arg

10

1.3.8 His

13

1.3.9 The Aromatic Residues: Phe, Tyr, and Trp

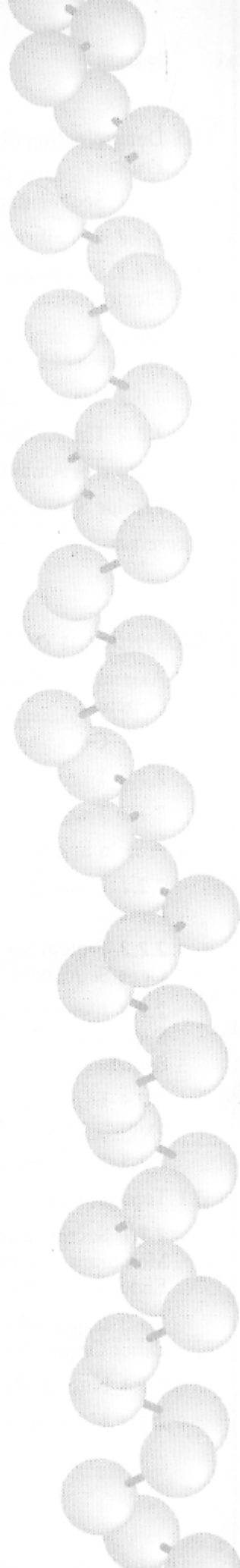
14

1.3.10 The Sulfur-Containing Residues: Met and Cys

17

1.4 Detection of Amino Acids, Peptides, and Proteins

20



1.5 Determination of the Sizes of Proteins	23	2.3.5 Bacterial Secreted and Membrane Proteins	77
1.5.1 Sedimentation Analysis	24	2.4 Posttranslational Covalent Modifications of Polypeptide Chains	78
1.5.2 Gel Filtration	24	2.4.1 Proteolytic Processing	78
1.5.3 SDS Polyacrylamide Gel Electrophoresis	26	2.4.2 Alteration of the Chain Termini	86
1.6 Determination of the Covalent Structures of Proteins	28	2.4.3 Glycosylation	91
1.6.1 Amino Acid Composition	28	2.4.4 Lipid Attachment	94
1.6.2 The Amino Acid Sequence	31	2.4.5 Sulfation	95
1.7 Nature of Amino Acid Sequences	43	2.4.6 γ -Carboxy-Glu Residues	95
1.8 Peptide Synthesis	43	2.4.7 Hydroxylation	96
<i>Exercises</i>	47	2.4.8 Phosphorylation	96
		2.4.9 ADP-Ribosylation	98
		2.4.10 Disulfide Bond Formation	98
		2.4.11 Common Nonenzymatic, Chemical Modifications	99
2 Biosynthesis of Proteins	49	2.5 Nonribosomal Biosynthesis of Unusual Peptides	100
2.1 Assembly of the Primary Structure	50	<i>Exercises</i>	102
2.1.1 Gene Structure	50		
2.1.2 Transcription	51		
2.1.3 Translation	53		
2.2 Protein Engineering	59	3 Evolutionary and Genetic Origins of Protein Sequences	105
2.2.1 Isolating the Gene for a Protein	59	3.1 Primordial Origins of Proteins	107
2.2.2 Protein Sequences from Gene Sequences	59	3.2 Evolutionary Divergence of Proteins	108
2.2.3 Identifying a Protein Specified by a Gene of Known Sequence	60	3.2.1 Homologous Genes and Proteins	108
2.2.4 Expressing a Cloned or Synthetic Gene	62	3.2.2 Mutations and Protein Structure	111
2.2.5 Site-Directed Mutagenesis	63	3.2.3 Genetic Divergence During Evolution	113
2.3 Topogenesis	64	3.3 Reconstructing Evolution from Contemporary Sequences	114
2.3.1 Secreted, Lysosomal, and Membrane Proteins, Via the Endoplasmic Reticulum	65	3.3.1 Variation among Species	114
2.3.2 Import of Proteins into Other Organelles	71	3.3.2 Variation within Species	125
2.3.3 Nuclear Proteins	75	3.4 Gene Rearrangements and the Evolution of Protein Complexity	127
2.3.4 Membrane Proteins	75	3.4.1 Products of Gene Duplications	127

3.4.2 Evolution of Metabolic Pathways	131	5 Conformational Properties of Polypeptide Chains	171
3.4.3 Protein Elongation by Intragene Duplication	132	5.1 Three-Dimensional Conformations	172
3.4.4 Gene Fusion and Division	132	5.2 Polypeptides as Random Polymers	173
3.4.5 Genetic Consequences of Duplicated Genes	134	5.2.1 Local Restrictions on Flexibility: The Ramachandran Plot	173
3.5 Using Genetics to Probe Protein Structure	134	5.2.2 Statistical Properties of Random Polypeptides	176
3.5.1 Selecting for Functional Mutations	135	5.2.3 Rates of Conformational Change	180
3.5.2 Simulation of Evolution	136	5.3 Regular Conformations of Polypeptides	182
<i>Exercises</i>	137	5.3.1 The α -Helix	182
4 Physical Interactions That Determine the Properties of Proteins	139	5.3.2 β -Sheets	186
4.1 The Physical Nature of Non-covalent Interactions	140	5.3.3 Other Regular Conformations	187
4.1.1 Short-Range Repulsions	140	5.4 Experimental Characterization of Polypeptides in Solution	189
4.1.2 Electrostatic Forces	142	5.4.1 Hydrodynamic Properties	189
4.1.3 Van der Waals Interactions	146	5.4.2 Spectral Properties	190
4.1.4 Hydrogen Bonds	147	5.5 Fibrous Proteins	193
4.2 The Proteins of Liquid Water and the Characteristics of Noncovalent Interactions in This Solvent	148	5.5.1 Silk Fibroin	193
4.2.1 Liquids	149	5.5.2 Coiled Coils	193
4.2.2 Water	150	5.5.3 Collagen Triple Helix	196
4.2.3 Aqueous Solutions	153	<i>Exercises</i>	198
4.3 The Hydrophobic Interaction	157	6 The Folded Conformations of Globular Proteins	201
4.3.1 The Hydrophobic Interaction in Model Systems	157	6.1 Three-Dimensional Structures by X-ray Diffraction	202
4.3.2 Hydrophobicities of Amino Acid Residues	160	6.1.1 Crystallizing Proteins	202
4.4 Intramolecular Interactions	162	6.1.2 Basic Principles of Diffraction	203
4.4.1 Effective Concentrations	163	6.1.3 Phase Determination	208
4.4.2 Multiple Interactions	165	6.1.4 Calculation of the Electron Density Map	210
4.4.3 Cooperativity of Multiple Interactions	165	6.1.5 Interpretation of the Electron Density Map	213
<i>Exercises</i>	167		

6.1.6 Refinement of the Model	214	7 Proteins in Solution and in Membranes	261
6.1.7 Rapid Diffraction Measurements	215	7.1 Physical and Chemical Properties of Soluble Proteins	261
6.1.8 Neutron Diffraction	216	7.1.1 Aqueous Solubility	262
6.2 The General Properties of Protein Structures	217	7.1.2 Hydrodynamic Properties in Aqueous Solution	264
6.2.1 The Tertiary Structure	217	7.1.3 Spectral Properties	270
6.2.2 Secondary Structure	221	7.1.4 Ionization	271
6.2.3 Reverse Turns	225	7.1.5 Chemical Properties	272
6.2.4 Supersecondary Structures	227	7.2 Proteins in Membranes	276
6.2.5 Interiors and Exteriors	227	7.2.1 Association with Membranes	277
6.2.6 Quaternary Structure	232	7.2.2 Structures of Integral Membrane Proteins	278
6.2.7 Flexibility Detected Crystallographically	236	7.2.3 Identifying Amino Acid Sequences Likely to Traverse Membranes	280
6.2.8 The Solvent	238	7.2.4 Dynamic Behavior in Membranes	280
6.3 Protein Structure Determination by Nuclear Magnetic Resonance Spectroscopy	238	7.3 Flexibility of Protein Structure	281
6.3.1 Nuclear Magnetic Resonance Spectra of Proteins	238	7.3.1 Hydrogen Exchange	282
6.3.2 Determining a Protein Structure by Nuclear Magnetic Resonance	243	7.3.2 Fluorescence Quenching and Depolarization	286
6.4 Proteins with Similar Folded Conformations	244	7.3.3 Rotations of Side Chains	286
6.4.1 Evolutionarily Related Proteins	244	7.4 Stability of the Folded Conformation	287
6.4.2 Conformational Similarity without Apparent Sequence Homology	249	7.4.1 Reversible Unfolding Transitions	287
6.4.3 Structural Homology within a Polypeptide Chain	251	7.4.2 Nature of the Unfolded State	291
6.5 Rationalization and Prediction of Protein Structure	253	7.4.3 Physical Basis For Protein Denaturation	292
6.5.1 Predicting Secondary Structure	255	7.4.4 Effects on Stability of Variation of the Primary Structure	303
6.5.2 Modeling Homologous Protein Structures	257	7.5 Mechanism of Protein Folding	309
6.5.3 De Novo Protein Design	258	7.5.1 Kinetic Analysis of Complex Reactions	309
<i>Exercises</i>	259	7.5.2 Kinetics of Unfolding	310

7.5.3 Kinetics of Refolding	311	8.4.4 Other Allosteric O ₂ -Binding Proteins	380
7.5.4 Folding Pathways	316	8.4.5 Negative Cooperativity	381
7.5.5 Folding of Large Proteins	321	<i>Exercises</i>	382
7.5.6 Biosynthetic Folding	323		
<i>Exercises</i>	325	9 Enzyme Catalysis	385
8 Interactions with Other Molecules	329	9.1 The Kinetics of Enzyme Action	386
8.1 Structures of Protein-Ligand Complexes	330	9.1.1 Steady-State Kinetics	386
8.1.1 The Difference Fourier Crystallographic Technique	330	9.1.2 Reactions on the Enzyme	392
8.1.2 NMR	333	9.2 Theories of Enzyme Catalysis	396
8.1.3 Chemical Methods of Determining Binding Sites	333	9.2.1 Rate Enhancements	396
8.1.4 General Properties of Ligand Binding Interactions	334	9.2.2 Transition-State Stabilization	402
8.2 Energetics and Dynamics of Binding	337	9.2.3 Transition-State Analogues	403
8.2.1 Binding Affinities	338	9.2.4 Catalytic Antibodies	406
8.2.2 Accounting for Relative Affinities	340	9.2.5 Substrate Specificity and Induced Fit	406
8.2.3 Rates of Binding and Dissociation	344	9.2.6 Testing Theories of Catalysis	410
8.2.4 Affinity Chromatography	346	9.3 Examples of Enzyme Mechanisms	413
8.3 Relationship between Protein Conformation and Binding	348	9.3.1 Tyrosyl tRNA Synthetase	413
8.3.1 Immunoglobulins	348	9.3.2 Proteases	417
8.3.2 DNA-Binding Proteins	355	9.3.3 Lysozyme	437
8.3.3 Nucleotide Binding	360	9.4 Regulation of Enzyme Activity	441
8.3.4 Very Small Ligands	361	9.4.1 Enzyme Function in Vivo	442
8.4 Allosteric Interactions between Binding Sites	367	9.4.2 Allosteric Regulation	444
8.4.1 Multiple Binding Sites and Interactions between Them	368	9.4.3 Reversible Covalent Modification	452
8.4.2 Allosteric Models	372	<i>Exercises</i>	459
8.4.3 The Allosteric Properties of Hemoglobin	374	10 Degradation	463
		10.1 Chemical Aging	464
		10.2 Protein Turnover in Vivo	465
		10.2.1 Factors That Determine the Rate of Degradation	466
		10.3 Mechanisms of Protein Degradation	468

10.3.1 Proteases Involved in Protein Turnover	468	<i>Appendix 2 References to Protein Structures Determined Crystallographically to High Resolution</i>	477
10.3.2 Lysosomes	469		
10.3.3 Ubiquitin-Mediated Pathway	470		
Exercises	472	Index	491
<i>Appendix 1 Major Protein and DNA Sequence Data Banks</i>	475		