

Contents

<i>Foreword</i>	<i>page</i> xiii
1 Introduction	1
1.1 Sources and roles of amino acids and peptides	1
1.2 Definitions	1
1.3 'Protein amino acids', alias 'the coded amino acids'	3
1.4 Nomenclature for 'the protein amino acids', alias 'the coded amino acids'	7
1.5 Abbreviations for names of amino acids and the use of these abbreviations to give names to polypeptides	7
1.6 Post-translational processing: modification of amino-acid residues within polypeptides	11
1.7 Post-translational processing: <i>in vivo</i> cleavages of the amide backbone of polypeptides	11
1.8 'Non-protein amino acids', alias 'non-proteinogenic amino acids' or 'non-coded amino acids'	11
1.9 Coded amino acids, non-natural amino acids and peptides in nutrition and food science and in human physiology	13
1.10 The geological and extra-terrestrial distribution of amino acids	15
1.11 Amino acids in archaeology and in forensic science	15
1.12 Roles for amino acids in chemistry and in the life sciences	16
1.12.1 Amino acids in chemistry	16
1.12.2 Amino acids in the life sciences	16
1.13 β - and higher amino acids	17
1.14 References	19
2 Conformations of amino acids and peptides	20
2.1 Introduction: the main conformational features of amino acids and peptides	20

 Contents

2.2	Configurational isomerism within the peptide bond	20
2.3	Dipeptides	26
2.4	Cyclic oligopeptides	26
2.5	Acyclic oligopeptides	27
2.6	Longer oligopeptides: primary, secondary and tertiary structure	27
2.7	Polypeptides and proteins: quaternary structure and aggregation	28
2.8	Examples of conformational behaviour; ordered and disordered states and transitions between them	29
2.8.1	The main categories of polypeptide conformation	29
2.8.1.1	One extreme situation	29
2.8.1.2	The other extreme situation	29
2.8.1.3	The general case	29
2.9	Conformational transitions for amino acids and peptides	30
2.10	References	31
3	Physicochemical properties of amino acids and peptides	32
3.1	Acid–base properties	32
3.2	Metal-binding properties of amino acids and peptides	34
3.3	An introduction to the routine aspects and the specialised aspects of the spectra of amino acids and peptides	35
3.4	Infrared (IR) spectrometry	36
3.5	General aspects of ultraviolet (UV) spectrometry, circular dichroism (CD) and UV fluorescence spectrometry	37
3.6	Circular dichroism	38
3.7	Nuclear magnetic resonance (NMR) spectroscopy	41
3.8	Examples of assignments of structures to peptides from NMR spectra and other data	43
3.9	References	46
4	Reactions and analytical methods for amino acids and peptides	48
	<i>Part I</i> Reactions of amino acids and peptides	48
4.1	Introduction	48
4.2	General survey	48
4.2.1	Pyrolysis of amino acids and peptides	49
4.2.2	Reactions of the amino group	49
4.2.3	Reactions of the carboxy group	49
4.2.4	Reactions involving both amino and carboxy groups	51
4.3	A more detailed survey of reactions of the amino group	51
4.3.1	<i>N</i> -Acylation	51
4.3.2	Reactions with aldehydes	52
4.3.3	<i>N</i> -Alkylation	53

 Contents

4.4	A survey of reactions of the carboxy group	53
4.4.1	Esterification	54
4.4.2	Oxidative decarboxylation	54
4.4.3	Reduction	54
4.4.4	Halogenation	55
4.4.5	Reactions involving amino and carboxy groups of α -amino acids and their <i>N</i> -acyl derivatives	55
4.4.6	Reactions at the α -carbon atom and racemisation of α -amino acids	55
4.4.7	Reactions of the amide group in acylamino acids and peptides	57
4.5	Derivatisation of amino acids for analysis	58
4.5.1	Preparation of <i>N</i> -acylamino acid esters and similar derivatives for analysis	58
4.6	References	60
<i>Part 2</i> Mass spectrometry in amino-acid and peptide analysis and in peptide-sequence determination		61
4.7	General considerations	61
4.7.1	Mass spectra of free amino acids	61
4.7.2	Mass spectra of free peptides	62
4.7.3	Negative-ion mass spectrometry	65
4.8	Examples of mass spectra of peptides	65
4.8.1	Electron-impact mass spectra (EIMS) of peptide derivatives	65
4.8.2	Finer details of mass spectra of peptides	68
4.8.3	Difficulties and ambiguities	69
4.9	The general status of mass spectrometry in peptide analysis	69
4.9.1	Specific advantages of mass spectrometry in peptide sequencing	70
4.10	Early methodology: peptide derivatisation	71
4.10.1	<i>N</i> -Terminal acylation and <i>C</i> -terminal esterification	71
4.10.2	<i>N</i> -Acylation and <i>N</i> -alkylation of the peptide bond	72
4.10.3	Reduction of peptides to 'polyamino-polyalcohols'	72
4.11	Current methodology: sequencing by partial acid hydrolysis, followed by direct MS analysis of peptide hydrolysates	72
4.11.1	Current methodology: instrumental variations	74
4.12	Conclusions	77
4.13	References	77

 Contents

<i>Part 3</i>	Chromatographic and related methods for the separation of mixtures of amino acids, mixtures of peptides and mixtures of amino acids and peptides	78
4.14	Separation of amino-acid and peptide mixtures	78
4.14.1	Separation principles	78
4.15	Partition chromatography; HPLC and GLC	80
4.16	Molecular exclusion chromatography (gel chromatography)	80
4.17	Electrophoretic separation and ion-exchange chromatography	82
4.17.1	Capillary zone electrophoresis (CZE)	83
4.18	Detection of separated amino acids and peptides	83
4.18.1	Detection of amino acids and peptides separated by HPLC and by other liquid-based techniques	84
4.18.2	Detection of amino acids and peptides separated by GLC	85
4.19	Thin-layer chromatography (planar chromatography; HPTLC)	86
4.20	Quantitative amino-acid analysis	86
4.21	References	87
<i>Part 4</i>	Immunoassays for peptides	87
4.22	Radioimmunoassays	87
4.23	Enzyme-linked immunosorbent assays (ELISAs)	88
4.24	References	90
<i>Part 5</i>	Enzyme-based methods for amino acids	90
4.25	Biosensors	90
4.26	References	90
5	Determination of the primary structure of peptides and proteins	91
5.1	Introduction	91
5.2	Strategy	92
5.3	Cleavage of disulphide bonds	96
5.4	Identification of the <i>N</i> -terminus and stepwise degradation	97
5.5	Enzymic methods for determining <i>N</i> -terminal sequences	105
5.6	Identification of <i>C</i> -terminal sequences	106
5.7	Enzymic determination of <i>C</i> -terminal sequences	107
5.8	Selective chemical methods for cleaving peptide bonds	107
5.9	Selective enzymic methods for cleaving peptide bonds	109
5.10	Determination of the positions of disulphide bonds	112
5.11	Location of post-translational modifications and prosthetic groups	114
5.12	Determination of the sequence of DNA	117
5.13	References	118

 Contents

6	Synthesis of amino acids	120
6.1	General	120
6.2	Commercial and research uses for amino acids	120
6.3	Biosynthesis: isolation of amino acids from natural sources	121
6.3.1	Isolation of amino acids from proteins	121
6.3.2	Biotechnological and industrial synthesis of coded amino acids	121
6.4	Synthesis of amino acids starting from coded amino acids other than glycine	122
6.5	General methods of synthesis of amino acids starting with a glycine derivative	123
6.6	Other general methods of amino acid synthesis	123
6.7	Resolution of DL-amino acids	125
6.8	Asymmetric synthesis of amino acids	127
6.9	References	129
7	Methods for the synthesis of peptides	130
7.1	Basic principles of peptide synthesis and strategy	130
7.2	Chemical synthesis and genetic engineering	132
7.3	Protection of α -amino groups	134
7.4	Protection of carboxy groups	135
7.5	Protection of functional side-chains	138
7.5.1	Protection of ϵ -amino groups	138
7.5.2	Protection of thiol groups	139
7.5.3	Protection of hydroxy groups	140
7.5.4	Protection of the guanidino group of arginine	141
7.5.5	Protection of the imidazole ring of histidine	142
7.5.6	Protection of amide groups	145
7.5.7	Protection of the thioether side-chain of methionine	145
7.5.8	Protection of the indole ring of tryptophan	146
7.6	Deprotection procedures	146
7.7	Enantiomerisation during peptide synthesis	146
7.8	Methods for forming peptide bonds	149
7.8.1	The acyl azide method	150
7.8.2	The use of acid chlorides and acid fluorides	151
7.8.3	The use of acid anhydrides	151
7.8.4	The use of carbodiimides	153
7.8.5	The use of reactive esters	153
7.8.6	The use of phosphonium and isouronium derivatives	155
7.9	Solid-phase peptide synthesis (SPPS)	156
7.10	Soluble-handle techniques	163
7.11	Enzyme-catalysed peptide synthesis and partial synthesis	164

 Contents

7.12	Cyclic peptides	168
7.12.1	Homodetic cyclic peptides	168
7.12.2	Heterodetic cyclic peptides	170
7.13	The formation of disulphide bonds	170
7.14	References	172
7.14.1	References cited in the text	172
7.14.2	References for background reading	173
8	Biological roles of amino acids and peptides	174
8.1	Introduction	174
8.2	The role of amino acids in protein biosynthesis	175
8.3	Post-translational modification of protein structures	178
8.4	Conjugation of amino acids with other compounds	182
8.5	Other examples of synthetic uses of amino acids	183
8.6	Important products of amino-acid metabolism	187
8.7	Glutathione	190
8.8	The biosynthesis of penicillins and cephalosporins	192
8.9	References	198
8.9.1	References cited in the text	198
8.9.2	References for background reading	199
9	Some aspects of amino-acid and peptide drug design	200
9.1	Amino-acid antimetabolites	200
9.2	Fundamental aspects of peptide drug design	201
9.3	The need for peptide-based drugs	202
9.4	The mechanism of action of proteinases and design of inhibitors	204
9.5	Some biologically active analogues of peptide hormones	210
9.6	The production of antibodies and vaccines	213
9.7	The combinatorial synthesis of peptides	215
9.8	The design of pro-drugs based on peptides	216
9.9	Peptide antibiotics	217
9.10	References	218
9.10.1	References cited in the text	218
9.10.2	References for background reading	218
	<i>Subject index</i>	220