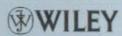




Pharmaceutical Biotechnology

Concepts and Applications

Gary Walsh



Contents

Pr	етас		xv
Ac	rony	ms	xvii
1	Pha	1	
	1.1	Introduction to pharmaceutical products	1
	1.2	Biopharmaceuticals and pharmaceutical biotechnology	1
	1.3	History of the pharmaceutical industry	2
	1.4	The age of biopharmaceuticals	3
	1.5	Biopharmaceuticals: current status and future prospects	8
		Further reading	11
2	Pro	tein structure	13
	2.1	Introduction	13
	2.2	Overview of protein structure	13
		2.2.1 Primary structure	15
		2.2.2 The peptide bond	18
		2.2.3 Amino acid sequence determination	19
		2.2.4 Polypeptide synthesis	22
	2.3	Higher level structure	23
		2.3.1 Secondary structure	23
		2.3.2 Tertiary structure	26
		2.3.3 Higher structure determination	26
	2.4	Protein stability and folding	27
		2.4.1 Structural prediction	28
	2.5	Protein post-translational modification	29
		2.5.1 Glycosylation	29
		2.5.2 Carboxylation and hydroxylation	33
		2.5.3 Sulfation and amidation	34
		Further reading	35
3	Ger	ne manipulation and recombinant DNA technology	37
	3.1	Introduction	37
	3.2	Nucleic acids: function and structure	38
		3.2.1 Genome and gene organization	41
		3.2.2 Nucleic acid purification	43
		3.2.3 Nucleic acid sequencing	45
	3.3	Recombinant production of therapeutic proteins	46
	3.4	Classical gene cloning and identification	47
		3.4.1 cDNA cloning	51
		3.4.2 Cloning via polymerase chain reaction	51

viii CONTENTS

		3.4.3	Expression vectors	53
			Protein engineering	53
		Further	reading	54
4	The	drug de	evelopment process	57
	4.1	Introduc		57
			ry of biopharmaceuticals	58
			act of genomics and related technologies upon drug discovery	59
	4.4		,	61
	4.5	Proteom		62
	4.6		al genomics	64
	4.7		cogenetics	65
			roduct characterization	66 67
	4.9	Patentin	*	68
			What is a patent and what is patentable?	68
	4.10		Patenting in biotechnology of biopharmaceuticals	70
	4.10		Oral delivery systems	70
			Pulmonary delivery	71
			Nasal, transmucosal and transdermal delivery systems	73
	۸ 11		cal studies	74
	4.12		cokinetics and pharmacodynamics	74
	7.12		Protein pharmacokinetics	75
			Tailoring of pharmacokinetic profile	77
			Protein mode of action and pharmacodynamics	79
	4.13			80
			Reproductive toxicity and teratogenicity	82
			Mutagenicity, carcinogenicity and other tests	83
			Clinical trials	84
		4.13.4	Clinical trial design	87
		4.13.5	Trial size design and study population	87
	4.14	The role	and remit of regulatory authorities	89
		4.14.1	The Food and Drug Administration	90
		4.14.2	The investigational new drug application	92
		4.14.3	→ '''	94
			European regulations	95
	•		National regulatory authorities	96
		4.14.6	The European Medicines Agency and the new EU drug	
			approval systems	96
			The centralized procedure	98
			Mutual recognition	100
		4.14.9	· · · · · · · · · · · · · · · · ·	100
		4.14.10	World harmonization of drug approvals	101
	4.15			101
		Further	reading	101
5	Sou		d upstream processing	105
	5.1	Introduc		105
	5.2		of biopharmaceuticals	105
			Escherichia coli as a source of recombinant, therapeutic proteins	105
		5.2.2	Expression of recombinant proteins in animal cell culture systems	109

CONTENTS ix

		5.2.3 Additional production systems	110
		5.2.3.1 Yeast	110
		5.2.3.2 Fungal production systems	111
		5.2.3.3 Transgenic animals	111
		5.2.3.4 Transgenic plants	116
		5.2.3.5 Insect cell-based systems	118
	5.3	Upstream processing	120
		5.3.1 Cell banking systems	121
		5.3.2 Microbial cell fermentation	124
		5.3.3 Mammalian cell culture systems	127
		Further reading	129
6	Dov	•	131
	6.1	Introduction	131
	6.2	Initial product recovery	134
	6.3	Cell disruption	134
	6.4	Removal of nucleic acid	136
	6.5	Initial product concentration	137
		6.5.1 Ultrafiltration	137
		6.5.2 Diafiltration	139
	6.6	Chromatographic purification	140
		6.6.1 Size-exclusion chromatography (gel filtration)	142
		6.6.2 Ion-exchange chromatography	142
		6.6.3 Hydrophobic interaction chromatography 6.6.4 Affinity chromatography	146
		6.6.5 Immunoaffinity purifications	148
		6.6.6 Protein A chromatography	150 150
		6.6.7 Lectin affinity chromatography	150
		6.6.8 Dye affinity chromatography	152
		6.6.9 Metal chelate affinity chromatography	153
		6.6.10 Chromatography on hydroxyapatite	154
		6.6.11 Chromatofocusing	155
	6.7	High-performance liquid chromatography of proteins	155
	6.8	Purification of recombinant proteins	157
	6.9	Final product formulation	159
		6.9.1 Some influences that can alter the biological activity of proteins	159
		6.9.1.1 Proteolytic degradation and alteration of sugar side-chains	160
		6.9.1.2 Protein deamidation	161
		6.9.1.3 Oxidation and disulfide exchange	162
		6.9.2 Stabilizing excipients used in final product formulations	164
		6.9.3 Final product fill	166
		6.9.4 Freeze-drying	168
		6.9.5 Labelling and packing	169
		Further reading	171
7	Pro	duct analysis	173
	7.1	Introduction	173
	7.2	Protein-based contaminants	173
	7.3	Removal of altered forms of the protein of interest from the product stream	175
		7.3.1 Product potency	175

x CONTENTS

		7.3.2	Determination of protein concentration	179			
	7.4	Detec	tion of protein-based product impurities	180			
		7.4.1	Capillary electrophoresis	182			
		7.4.2	High-performance liquid chromatography	183			
			,	184			
	7.5		nological approaches to detection of contaminants	185			
			Amino acid analysis	185			
			Peptide mapping	186			
			N-terminal sequencing	188			
			Analysis of secondary and tertiary structure	188			
	7.6		oxin and other pyrogenic contaminants	189			
		7.6.1	,	191			
			Pyrogen detection	191			
		7.6.3		195			
		7.6.4		196			
			Viral assays	198			
			Miscellaneous contaminants Validation studies	199			
				199 202			
		rurtne	er reading	202			
8	The	cyto	kines: The interferon family	205			
	8.1	Cytok	ines	205			
				210			
				211			
	8.2		nterferons	212			
			The biochemistry of interferon- $lpha$	213			
			Interferon-β	214			
		8.2.3	•	214			
		8.2.4	3	214			
		8.2.5	•	215			
		8.2.6		215			
		8.2.7		218			
		8.2.8	•	219			
	0.3	8.2.9	, ,	221			
	8.3	8.3.1	feron biotechnology Production and medical uses of interferon-α	224 226			
			Medical uses of interferon-β	220			
		8.3.3	-	232			
			Interferon toxicity	234			
		8.3.5	Additional interferons	235			
	8.4	Concl		236			
	0.4		er reading	237			
9	Րս+	akina	se. Intarlauking and tumour nacrocic factor	241			
7	9.1	ytokines: Interleukins and tumour necrosis factor .1 Introduction					
	9.2		leukin-2	241 242			
	J.L		Interleukin-2 production	246			
			Interleukin-2 and cancer treatment	246			
			Interleukin-2 and infectious diseases	248			
			Safety issues	249			
			Inhihition of interleukin-2 activity	240			

CONTENTS xi

	9.3	Interleukin-1 9.3.1 The biological activities of interleukin-1 9.3.2 Interleukin-1 biotechnology	251 252 253		
	9.4	Interleukin-11	254		
	9.5	Tumour necrosis factors	255		
		9.5.1 Tumour necrosis factor biochemistry	255		
		9.5.2 Biological activities of tumour necrosis factor- α	256		
		9.5.3 Immunity and inflammation	257		
		9.5.4 Tumour necrosis factor receptors	258		
		9.5.5 Tumour necrosis factor: therapeutic aspects	260		
		Further reading	262		
10	Grov	vth factors	265		
	10.1	Introduction	265		
	10.2	Haematopoietic growth factors	265		
		10.2.1 The interleukins as haemopoietic growth factors	268		
		10.2.2 Granulocyte colony-stimulating factor	269		
		10.2.3 Macrophage colony-stimulating factor	269		
		10.2.4 Granulocyte macrophage colony-stimulating factor	270		
		10.2.5 Clinical application of colony-stimulating factors	270		
		10.2.6 Erythropoietin	272		
		10.2.6.1 Therapeutic applications of erythropoietin	274		
		10.2.6.2 Chronic disease and cancer chemotherapy	278		
		10.2.7 Thrombopoietin	278		
	10.3	Growth factors and wound healing	279		
		10.3.1 Insulin-like growth factors	280		
		10.3.2 Insulin-like growth factor biological effects	281		
		10.3.3 Epidermal growth factor	282		
		10.3.4 Platelet-derived growth factor	283		
		10.3.5 Fibroblast growth factors	284		
		10.3.6 Transforming growth factors	284		
		10.3.7 Neurotrophic factors	286		
		Further reading	287		
11	The	rapeutic hormones	291		
	11.1		291		
	11.2	Insulin	291		
		11.2.1 Diabetes meilitus	292		
		11.2.2 The insulin molecule	293		
		11.2.3 The insulin receptor and signal transduction	294		
		11.2.4 Insulin production	294		
		11.2.5 Production of human insulin by recombinant DNA technology	297		
		11.2.6 Formulation of insulin products	297		
		11.2.7 Engineered insulins	301		
		11.2.8 Additional means of insulin administration	304 305		
	11.3	3			
	11.4	Human growth hormone	307		
		11.4.1 The growth hormone receptor	307		
		11.4.2 Biological effects of growth hormone	308		
		11.4.3 Therapeutic uses of growth hormone	309		

xii CONTENTS

	11.5	The go	nadotrophins	310		
		11.5.1	Follicle-stimulating hormone, luteinizing hormone			
			and human chorionic gonadotrophin	311		
		11.5.2	Pregnant mare serum gonadotrophin	315		
		11.5.3	The inhibins and activins	315		
	11.6	Medica	l and veterinary applications of gonadotrophins	319		
		11.6.1	Sources and medical uses of follicle-stimulating hormone,			
			luteinizing hormone and human chorionic gonadotrophin	319		
		11.6.2	Recombinant gonadotrophins	320		
		11.6.3	Veterinary uses of gonadotrophins	321		
	11.7	Additio	onal recombinant hormones now approved	323		
	11.8	Conclu	sion	325		
		Furthe	r reading	325		
12	Rece	ombina	int blood products and therapeutic enzymes	329		
	12.1	Introd	uction	329		
	12.2	Haemo		329		
		12.2.1	The coagulation pathway	330		
		12.2.2	Terminal steps of coagulation pathway	332		
		12.2.3	Clotting disorders	334		
		12.2.4	Factor VIII and haemophilia	335		
		12.2.5	Production of factor VIII	336		
			Factors IX, IIVa and XIII	339		
	12.3	Antico	agulants	340		
			Hirudin	342		
			Antithrombin	344		
	12.4		polytic agents	345		
			Tissue plasminogen activator	346		
		12.4.2		348		
		12.4.3	3 1 3 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	348		
		12.4.4	'	350		
		12.4.5		350		
			Staphylokinase	351		
			$lpha_1$ -Antitrypsin	353		
		12.4.8	Albumin	354		
	12.5		es of therapeutic value	355		
			Asparaginase	355		
		12.5.2		357		
		12.5.3		359		
		12.5.4	· · · · · · · · · · · · · · · · · · ·	360		
		12.5.5	Superoxide dismutase	363		
		12.5.6	Debriding agents	364		
		12.5.7	Digestive aids	364		
		Furthe	r reading	366		
13			s, vaccines and adjuvants	371		
	13.1					
	13.2		onal polyclonal antibody preparations	371		
	13.3		lonal antibodies	374		
		13.3.1	Antibody screening: phage display technology	376		
		13.3.2	Therapeutic application of monoclonal antibodies	378		

CONTENTS xiii

		13.3.3	Tumour im	ımunology	379
			13.3.3.1	Antibody-based strategies for tumour	
				detection/destruction	383
				Drug-based tumour immunotherapy	386
				First-generation anti-tumour antibodies:	
				clinical disappointment	388
		13.3.4		sociated antigens	389
		13.3.5		ity of murine monoclonals	391
		13.3.6		and humanized antibodies	392
			Antibody 1		394
				l therapeutic applications of monoclonal antibodies	395
	13.4		technolog		396
				l vaccine preparations	396
		-+		Attenuated, dead or inactivated bacteria	398
				Attenuated and inactivated viral vaccines	399
				Toxoids and antigen-based vaccines	399
		13.4.2		t of genetic engineering on vaccine technology	400
		13.4.3			402
		13.4.4			403
		13.4.5		ent of an AIDS vaccine	407
				s associated with vaccine development	409
		13.4.7		ines in clinical trials	409
		13,4.8			410
				ant veterinary vaccines	411
	13.5		nt technolo		412
				mode of action	413
				sed adjuvants	413
		13.5.3		emulsion adjuvants	414
		13.5.4		pacterial products as adjuvants	414
		13.5.5		. adjuvants	415
		Further	reading	•	416
14	Nucl	leic-aci	d- and ce	ell-based therapeutics	419
	14.1	Introdu	ıction		419
	14.2	Gene th	nerapy		419
		14.2.1	Basic appr	oach to gene therapy	420
		14.2.2	Some addi	tional questions	423
	14.3	Vectors	used in ge	ne therapy	424
		14.3.1	Retroviral	vectors	424
		14.3.2	Adenovira	l and additional viral-based vectors	428
		14.3.3	Manufactu	re of viral vectors	431
		14.3.4	Non-viral	vectors	432
		14.3.5	Manufactu	re of plasmid DNA	436
	14.4	Gene th	erapy and (genetic disease	438
	14.5	Gene th	erapy and o	cancer	441
	14.6	Gene th	erapy and ι	AIDS	444
		14.6.1		d vaccines	444
		14.6.2		apy: some additional considerations	445
	14.7		ise technolo		445
		14.7.1		oligonucleotides and their mode of action	446
		14.7.2	Uses, adva	ntages and disadvantages of 'oligos'	448

ITENT:
П

Index

14.8	Oligonucleotide pharmacokinetics and delivery	45
	14.8.1 Manufacture of oligos	45
	14.8.2 Additional antigene agents: RNA interference and ribozymes	45
14.9	Aptamers	45
14.10	Cell- and tissue-based therapies	45
	14.10.1 Stem cells	45
	14.10.2 Adult stem cells	45
14.11	Conclusion	46
	Further reading	46
	-	

465