Contents

Preface xv
Acronyms xvii

1 Pharmaceuticals, biologics and biopharmaceuticals 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 Protein structure 13
  2.1 Introduction 13
  2.2 Overview of protein structure
    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
    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
    2.4.1 Structural prediction 27
  2.5 Protein post-translational modification
    2.5.1 Glycosylation 29
    2.5.2 Carboxylation and hydroxylation 33
    2.5.3 Sulfation and amidation 34
    Further reading 35

3 Gene manipulation and recombinant DNA technology 37
  3.1 Introduction 37
  3.2 Nucleic acids: function and structure
    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
    3.4.1 cDNA cloning 51
    3.4.2 Cloning via polymerase chain reaction 51
### Contents

3.4.3 Expression vectors 53  
3.4.4 Protein engineering 53  
Further reading 54  

4 The drug development process 57  

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>57</td>
</tr>
<tr>
<td>4.2 Discovery of biopharmaceuticals</td>
<td>58</td>
</tr>
<tr>
<td>4.3 The impact of genomics and related technologies upon drug discovery</td>
<td>59</td>
</tr>
<tr>
<td>4.4 Gene chips</td>
<td>61</td>
</tr>
<tr>
<td>4.5 Proteomics</td>
<td>62</td>
</tr>
<tr>
<td>4.6 Structural genomics</td>
<td>64</td>
</tr>
<tr>
<td>4.7 Pharmacogenetics</td>
<td>65</td>
</tr>
<tr>
<td>4.8 Initial product characterization</td>
<td>66</td>
</tr>
<tr>
<td>4.9 Patenting</td>
<td>67</td>
</tr>
<tr>
<td>4.9.1 What is a patent and what is patentable?</td>
<td>68</td>
</tr>
<tr>
<td>4.9.2 Patenting in biotechnology</td>
<td>68</td>
</tr>
<tr>
<td>4.10 Delivery of biopharmaceuticals</td>
<td>70</td>
</tr>
<tr>
<td>4.10.1 Oral delivery systems</td>
<td>70</td>
</tr>
<tr>
<td>4.10.2 Pulmonary delivery</td>
<td>71</td>
</tr>
<tr>
<td>4.10.3 Nasal, transmucosal and transdermal delivery systems</td>
<td>73</td>
</tr>
<tr>
<td>4.11 Preclinical studies</td>
<td>74</td>
</tr>
<tr>
<td>4.12 Pharmacokinetics and pharmacodynamics</td>
<td>74</td>
</tr>
<tr>
<td>4.12.1 Protein pharmacokinetics</td>
<td>75</td>
</tr>
<tr>
<td>4.12.2 Tailoring of pharmacokinetic profile</td>
<td>77</td>
</tr>
<tr>
<td>4.12.3 Protein mode of action and pharmacodynamics</td>
<td>79</td>
</tr>
<tr>
<td>4.13 Toxicity studies</td>
<td>80</td>
</tr>
<tr>
<td>4.13.1 Reproductive toxicity and teratogenicity</td>
<td>82</td>
</tr>
<tr>
<td>4.13.2 Mutagenicity, carcinogenicity and other tests</td>
<td>83</td>
</tr>
<tr>
<td>4.13.3 Clinical trials</td>
<td>84</td>
</tr>
<tr>
<td>4.13.4 Clinical trial design</td>
<td>87</td>
</tr>
<tr>
<td>4.13.5 Trial size design and study population</td>
<td>87</td>
</tr>
<tr>
<td>4.14 The role and remit of regulatory authorities</td>
<td>89</td>
</tr>
<tr>
<td>4.14.1 The Food and Drug Administration</td>
<td>90</td>
</tr>
<tr>
<td>4.14.2 The investigational new drug application</td>
<td>92</td>
</tr>
<tr>
<td>4.14.3 The new drug application</td>
<td>94</td>
</tr>
<tr>
<td>4.14.4 European regulations</td>
<td>95</td>
</tr>
<tr>
<td>4.14.5 National regulatory authorities</td>
<td>96</td>
</tr>
<tr>
<td>4.14.6 The European Medicines Agency and the new EU drug approval systems</td>
<td>96</td>
</tr>
<tr>
<td>4.14.7 The centralized procedure</td>
<td>98</td>
</tr>
<tr>
<td>4.14.8 Mutual recognition</td>
<td>100</td>
</tr>
<tr>
<td>4.14.9 Drug registration in Japan</td>
<td>100</td>
</tr>
<tr>
<td>4.14.10 World harmonization of drug approvals</td>
<td>101</td>
</tr>
<tr>
<td>4.15 Conclusion</td>
<td>101</td>
</tr>
<tr>
<td>Further reading</td>
<td>101</td>
</tr>
</tbody>
</table>

5 Sources and upstream processing 105  

<table>
<thead>
<tr>
<th>Section</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Introduction</td>
<td>105</td>
</tr>
<tr>
<td>5.2 Sources of biopharmaceuticals</td>
<td>105</td>
</tr>
<tr>
<td>5.2.1 Escherichia coli as a source of recombinant, therapeutic proteins</td>
<td>105</td>
</tr>
<tr>
<td>5.2.2 Expression of recombinant proteins in animal cell culture systems</td>
<td>109</td>
</tr>
</tbody>
</table>
## 5.2.3 Additional production systems
- 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
- 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 Downstream processing

### 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
- 6.5.1 Ultrafiltration 137
- 6.5.2 Diafiltration 139

### 6.6 Chromatographic purification
- 6.6.1 Size-exclusion chromatography (gel filtration) 142
- 6.6.2 Ion-exchange chromatography 142
- 6.6.3 Hydrophobic interaction chromatography 146
- 6.6.4 Affinity chromatography 148
- 6.6.5 Immunoaffinity purifications 150
- 6.6.6 Protein A chromatography 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
- 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 Product analysis

### 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
CONTENTS

9.3 Interleukin-1 251
9.3.1 The biological activities of interleukin-1 252
9.3.2 Interleukin-1 biotechnology 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 Growth 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 Therapeutic hormones 291
11.1 Introduction 291
11.2 Insulin 291
11.2.1 Diabetes mellitus 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
11.3 Glucagon 305
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
CONTENTS

11.5 The gonadotrophins 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 Medical 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 Additional recombinant hormones now approved 323
11.8 Conclusion 325
Further reading 325

12 Recombinant blood products and therapeutic enzymes 329
  12.1 Introduction 329
  12.2 Haemostasis 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
    12.2.6 Factors IX, IIVa and XIII 339
  12.3 Anticoagulants 340
    12.3.1 Hirudin 342
    12.3.2 Antithrombin 344
  12.4 Thrombolytic agents 345
    12.4.1 Tissue plasminogen activator 346
    12.4.2 First-generation tissue plasminogen activator 348
    12.4.3 Engineered tissue plasminogen activator 348
    12.4.4 Streptokinase 350
    12.4.5 Urokinase 350
    12.4.6 Staphylokinase 351
    12.4.7 α1-Antitrypsin 353
    12.4.8 Albumin 354
  12.5 Enzymes of therapeutic value 355
    12.5.1 Asparaginase 355
    12.5.2 DNase 357
    12.5.3 Glucocerebrosidase 359
    12.5.4 α-Galactosidase, urate oxidase and laronidase 360
    12.5.5 Superoxide dismutase 363
    12.5.6 Debriding agents 364
    12.5.7 Digestive aids 364
  Further reading 366

13 Antibodies, vaccines and adjuvants 371
  13.1 Introduction 371
  13.2 Traditional polyclonal antibody preparations 371
  13.3 Monoclonal antibodies 374
    13.3.1 Antibody screening: phage display technology 376
    13.3.2 Therapeutic application of monoclonal antibodies 378
## CONTENTS

13.3.3 Tumour immunology 379

13.3.3.1 Antibody-based strategies for tumour detection/destruction 383

13.3.3.2 Drug-based tumour immunotherapy 386

13.3.3.3 First-generation anti-tumour antibodies: clinical disappointment 388

13.3.4 Tumour-associated antigens 389

13.3.5 Antigenicity of murine monoclonals 391

13.3.6 Chimaeric and humanized antibodies 392

13.3.7 Antibody fragments 394

13.3.8 Additional therapeutic applications of monoclonal antibodies 395

13.4 Vaccine technology 396

13.4.1 Traditional vaccine preparations 396

13.4.1.1 Attenuated, dead or inactivated bacteria 398

13.4.1.2 Attenuated and inactivated viral vaccines 399

13.4.1.3 Toxoids and antigen-based vaccines 399

13.4.2 The impact of genetic engineering on vaccine technology 400

13.4.3 Peptide vaccines 402

13.4.4 Vaccine vectors 403

13.4.5 Development of an AIDS vaccine 407

13.4.6 Difficulties associated with vaccine development 409

13.4.7 AIDS vaccines in clinical trials 409

13.4.8 Cancer vaccines 410

13.4.9 Recombinant veterinary vaccines 411

13.5 Adjuvant technology 412

13.5.1 Adjuvant mode of action 413

13.5.2 Mineral-based adjuvants 413

13.5.3 Oil-based emulsion adjuvants 414

13.5.4 Bacteria/bacterial products as adjuvants 414

13.5.5 Additional adjuvants 415

Further reading 416

14 Nucleic-acid- and cell-based therapeutics 419

14.1 Introduction 419

14.2 Gene therapy 419

14.2.1 Basic approach to gene therapy 420

14.2.2 Some additional questions 423

14.3 Vectors used in gene therapy 424

14.3.1 Retroviral vectors 424

14.3.2 Adenoviral and additional viral-based vectors 428

14.3.3 Manufacture of viral vectors 431

14.3.4 Non-viral vectors 432

14.3.5 Manufacture of plasmid DNA 436

14.4 Gene therapy and genetic disease 438

14.5 Gene therapy and cancer 441

14.6 Gene therapy and AIDS 444

14.6.1 Gene-based vaccines 444

14.6.2 Gene therapy: some additional considerations 445

14.7 Antisense technology 445

14.7.1 Antisense oligonucleotides and their mode of action 446

14.7.2 Uses, advantages and disadvantages of 'oligos' 448
CONTENTS

14.8 Oligonucleotide pharmacokinetics and delivery 450
   14.8.1 Manufacture of oligos 451
   14.8.2 Additional antigene agents: RNA interference and ribozymes 451
14.9 Aptamers 453
14.10 Cell- and tissue-based therapies 453
   14.10.1 Stem cells 457
   14.10.2 Adult stem cells 459
14.11 Conclusion 460
Further reading 460

Index 465