Pharmaceutical Formulation
Development of Peptides and Proteins

Edited by
SVEN FROKJAER AND LARS HOVGAARD
## Contents

<table>
<thead>
<tr>
<th>List of figures</th>
<th>page xi</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of tables</td>
<td>xiii</td>
</tr>
<tr>
<td>Contributors</td>
<td>xv</td>
</tr>
<tr>
<td>Preface</td>
<td>xvii</td>
</tr>
</tbody>
</table>

### 1 Peptide Synthesis

**Bernard A. Moss**

1.1 Introduction                                      | 1        |
1.2 Chemical synthesis of peptides                   | 2        |
1.2.1 Solution and solid phase peptide synthesis     | 4        |
1.2.2 Large-scale peptide synthesis                  | 6        |
1.3 Concluding remarks                                | 10       |
References and additional sources                    | 10       |

### 2 Basics in Recombinant DNA Technology

**Nanni Din and Jan Engberg**

2.1 Introduction                                      | 12       |
2.2 General methods in gene technology                | 13       |
2.2.1 DNA cloning tools                               | 13       |
2.2.2 Cloning of cDNA                                 | 15       |
2.2.3 PCR cloning and DNA database mining             | 16       |
2.3 Expression of recombinant proteins                | 18       |
2.3.1 Transcription, translation and protein modifications | 18   |
2.3.2 Choice of expression system                     | 19       |
2.4 Protein design                                    | 22       |
2.4.1 Protein variants                                | 22       |
2.4.2 Protein chimeras                                | 24       |
2.4.3 Epitope display libraries                       | 24       |
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 Recombinant protein therapeutics – status and future trends</td>
<td>25</td>
</tr>
<tr>
<td>References</td>
<td>27</td>
</tr>
<tr>
<td>3 Protein Purification</td>
<td>29</td>
</tr>
<tr>
<td>Lars Hovgaard, Lars Skriver and Sven Frokjaer</td>
<td></td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>29</td>
</tr>
<tr>
<td>3.2 Fractionation strategies</td>
<td>30</td>
</tr>
<tr>
<td>3.2.1 Initial fractionation step</td>
<td>30</td>
</tr>
<tr>
<td>3.2.2 Intermediate purification step</td>
<td>31</td>
</tr>
<tr>
<td>3.2.3 Final polishing step</td>
<td>32</td>
</tr>
<tr>
<td>3.2.4 The finished product</td>
<td>32</td>
</tr>
<tr>
<td>3.3 Protein stability in downstream processing</td>
<td>33</td>
</tr>
<tr>
<td>3.3.1 Protein conformation stability</td>
<td>33</td>
</tr>
<tr>
<td>3.3.2 Protein instability</td>
<td>34</td>
</tr>
<tr>
<td>3.3.3 Essential process-related parameters</td>
<td>37</td>
</tr>
<tr>
<td>References</td>
<td>38</td>
</tr>
<tr>
<td>4 Peptide and Protein Characterization</td>
<td>41</td>
</tr>
<tr>
<td>Miroslav Baudys and Sung Wan Kim</td>
<td></td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>41</td>
</tr>
<tr>
<td>4.2 Chromatography</td>
<td>43</td>
</tr>
<tr>
<td>4.2.1 Reversed phase chromatography</td>
<td>43</td>
</tr>
<tr>
<td>4.2.2 Hydrophobic interaction chromatography</td>
<td>46</td>
</tr>
<tr>
<td>4.2.3 Ion-exchange chromatography</td>
<td>47</td>
</tr>
<tr>
<td>4.2.4 Size-exclusion chromatography</td>
<td>47</td>
</tr>
<tr>
<td>4.3 Electrophoresis</td>
<td>48</td>
</tr>
<tr>
<td>4.3.1 Gel electrophoresis</td>
<td>48</td>
</tr>
<tr>
<td>4.3.2 Two-dimensional gel electrophoresis</td>
<td>50</td>
</tr>
<tr>
<td>4.3.3 Capillary electrophoresis</td>
<td>50</td>
</tr>
<tr>
<td>4.4 Structural characterization</td>
<td>51</td>
</tr>
<tr>
<td>4.4.1 Primary structure</td>
<td>52</td>
</tr>
<tr>
<td>4.4.2 Mass spectrometry</td>
<td>53</td>
</tr>
<tr>
<td>4.5 Secondary and tertiary structure</td>
<td>55</td>
</tr>
<tr>
<td>4.5.1 Absorption and fluorescence spectroscopy</td>
<td>56</td>
</tr>
<tr>
<td>4.5.2 Circular dichroism spectroscopy</td>
<td>57</td>
</tr>
<tr>
<td>4.5.3 Infrared spectroscopy</td>
<td>58</td>
</tr>
<tr>
<td>4.5.4 Other methods</td>
<td>59</td>
</tr>
<tr>
<td>4.6 Conclusion</td>
<td>60</td>
</tr>
<tr>
<td>References</td>
<td>60</td>
</tr>
<tr>
<td>5 Chemical Pathways of Peptide and Protein Degradation</td>
<td>70</td>
</tr>
<tr>
<td>Chimanlall Goolcharran, Mehrnaz Khosravi and Ronald T. Borchardt</td>
<td></td>
</tr>
<tr>
<td>5.1 Introduction</td>
<td>70</td>
</tr>
<tr>
<td>5.2 Hydrolytic pathways</td>
<td>71</td>
</tr>
<tr>
<td>5.2.1 Deamidation of Asn and Gln residues</td>
<td>71</td>
</tr>
<tr>
<td>5.2.2 Degradation of Asp residues</td>
<td>75</td>
</tr>
</tbody>
</table>
5.2.3 Degradation of N-terminal sequences containing penultimate Pro residues via diketopiperazine formation 78

5.3 Oxidation pathways
  5.3.1 Autooxidation 80
  5.3.2 Metal-catalysed oxidation 80
  5.3.3 Photooxidation 82
  5.3.4 Strategies to prevent oxidation 83

5.4 Other chemical pathways
  5.4.1 β-Elimination reactions 84
  5.4.2 Disulphide exchange reactions 84

5.5 Conclusion 85

References 86

6 Physical Stability of Proteins

Jens Brange

6.1 Introduction 89

6.2 Protein structure
  6.2.1 Stabilizing interactions 92
  6.2.2 Role of water in structure and stability 93

6.3 Protein destabilization (denaturation)
  6.3.1 Unfolding intermediates (molten globule) 95
  6.3.2 Temperature-induced changes 98
  6.3.3 Influence of pH 98
  6.3.4 Influence of pressure 99

6.4 Aggregation and precipitation
  6.4.1 Mechanisms of aggregation 100
  6.4.2 Precipitation and fibrillation phenomena 102
  6.4.3 Factors influencing aggregation and precipitation 105

6.5 Surface adsorption 106

6.6 Solid phase stability
  6.6.1 Lyophilization-induced aggregation 107

6.7 Stabilization of protein drugs
  6.7.1 Stabilization strategies 108

References 109

7 Peptides and Proteins as Parenteral Suspensions: an Overview of Design, Development, and Manufacturing Considerations

Michael R. DeFelippis and Michael J. Akers

7.1 Introduction and scope 113

7.2 Rationale for suspension development 114

7.3 Types of suspensions and particle formation
  7.3.1 In situ particle formation 116
  7.3.2 Combination of particles and vehicle 122

7.4 Excipient selection 124

7.5 General requirements for suspension products 126

7.6 Testing and optimization of chemical, physical, and microbiological properties 127
7.6 Chemical properties
7.6.1 Chemical properties
7.6.2 Physical properties
7.6.3 Microbiological properties
7.7 Techniques for characterizing and optimizing suspensions
7.8 Suspension manufacture
7.8.1 Scale-up
7.8.2 Manufacturing controls: special considerations for peptide and protein suspensions
7.9 Other related systems
7.10 Conclusions
Acknowledgements
References

8 Peptides and Proteins as Parenteral Solutions
Michael J. Akers and Michael R. DeFelippis
8.1 Overview and introduction
8.2 Optimizing hydrolytic stability
8.2.1 Buffers
8.2.2 Ionic strength
8.3 Optimizing oxidative stability
8.3.1 Antioxidants
8.3.2 Chelating agents
8.3.3 Inert gases
8.3.4 Packaging and oxidation
8.3.5 Other chemical stabilizers
8.4 Optimizing physical stability
8.4.1 Denaturation
8.4.2 Protein aggregation
8.4.3 Adsorption
8.4.4 Precipitation
8.4.5 Surfactants
8.4.6 Cyclodextrins
8.4.7 Albumin
8.4.8 Other physical complexing/stabilizing agents
8.5 Optimizing microbiological activity: antimicrobial preservatives (APs)
8.6 Osmolality (tonicity) agents
8.7 Packaging
8.8 Processing
8.9 Conclusion
References

9 Roles of Protein Conformation and Glassy State in the Storage Stability of Dried Protein Formulations
John F. Carpenter, Lotte Kreilgaard, S. Dean Allison and Theodore W. Randolph
9.1 Introduction
9.2 Infrared spectroscopy to study protein secondary structure
9.3 Physical factors affecting storage stability of dried protein formulations 181
9.4 Summary and conclusions 186
Acknowledgements 186
References 186

10 Peptide and Protein Drug Delivery Systems for Non-parenteral Routes of Administration 189
Mette Ingemann, Sven Frokjaer, Lars Hovgaard and Helle Brøndsted

10.1 Introduction 189
10.2 Non-parenteral routes of delivery for peptides and proteins 189
10.2.1 Barriers to non-parenteral administration of peptides and proteins 191
10.2.2 General approaches to bypass enzymatic and absorption barriers 192
10.3 Formulation principles for peptides and proteins 194
10.3.1 Entrapment and encapsulation 194
10.3.2 Covalent binding 198
10.4 Immobilized proteins intended for local effect in the GI tract – a case study 200
10.4.1 Oral enzyme supplementation therapy – phenylalanine ammonia-lyase 200
10.5 Future perspectives 202
10.6 Summary 203
References 203

11 Peptide and Protein Derivatives 206
Gitte Juel Friis

11.1 Introduction 206
11.2 4-Imidazolidinone prodrugs 207
11.3 Prodrugs of TRH 209
11.4 Derivatives of desmopressin 210
11.5 Derivatives of insulin 212
11.6 Cyclic prodrugs 213
11.7 Conclusions 214
References 215

12 Chemical and Pharmaceutical Documentation 220
Karen Fich and Deirdre Mannion

12.1 Introduction 220
12.2 Composition 221
12.3 Method of manufacture 222
12.4 Control of starting materials 222
12.4.1 Active substances 222
12.4.2 Excipients 226
12.4.3 Packaging materials 226
12.5 Intermediate products  
12.6 Control tests on the finished product  
12.7 Stability 
  12.7.1 Expert report  

References  

Index