

R. Kellner, F. Lottspeich,
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Microcharacterization of Proteins



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Preface

In June 1994 we organized the first meeting called "Mikromethoden in der Proteinchemie" at the Max Planck Institute for Biochemistry in Martinsried. Attendance was large, indicating an apparently broad-based interest in the detailed description of aspects of protein chemistry - perhaps because proteins are becoming more and more important in a variety of different disciplines. In selecting the presentations we focused on the most important methods, with descriptions of established ones as well as new ones. The speakers were experienced workers in the field, who made the conference attractive to interested learners and established 'protein people' alike. It was hoped that this would initiate lively exchange and dialogue between all participants. The chapters presented in this book are the written outlines of the spoken presentations and try to combine basic explanation with detailed descriptions of optimized procedures for sample handling at the micro level. Thanks to the help of all the authors, we are now able to offer this book to a wider public, and are very pleased of the chance to pass on various tips and ideas to those who need them. We should like to see this book become a valuable tool for anyone seeking to gain access to analytical problems in protein chemistry.

Heidelberg, Martinsried and Bochum. July 1994

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Contents

Section I: Overview

I.1 Microcharacterization of Proteins 3

Friedrich Lottspeich

- 1 General Aspects 3
- 2 From a Cell to a Protein Sequence 5
- 3 Future Trends 8

Section II: Sample Preparation

II.1 Chemical and Enzymatic Fragmentation of Proteins 11

Roland Kellner

- 1 Strategy 11
- 2 Denaturation, Reduction and Alkylation 13
- 3 Enzymatic Fragmentation 15
 - 3.1 Enzymes 17
 - 3.2 Practical Considerations 17
 - 4 Chemical Fragmentation 19
 - 4.1 Cyanogen Bromide Cleavage 19
 - 4.2 Partial Acid Hydrolysis 22
 - 4.3 Hydroxylamine Cleavage of Asn-Gly Bonds 22
 - 4.4 Cleavage at Tryptophan 24
 - 4.5 Cleavage at Cysteine 24
 - 5 References 25

II.2 Microseparation Techniques I: High Performance Liquid Chromatography 29

Maria Serwe and Helmut E. Meyer

- 1 Introduction 29
- 2 Getting Started 31
 - 2.1 Solvents 31
 - 2.2 Pump 32
 - 2.3 Pre-Column Split 33
 - 2.4 Sample Preparation 33
 - 2.5 Injector 33

- 2.6 Tubings 34
- 2.7 In-Line Filter, Guard Column 34
- 2.8 Analytical Column 34
- 2.9 Elution 37
- 2.10 Detection 37
- 2.11 Fractionation 38
- 3 Applications 39
- 4 References 44

II.3 Microseparation Techniques II: Analysis of Peptides and Proteins by Capillary Electrophoresis 47

Christine Schwer

- 1 Introduction 47
- 2 Theory 47
- 2.1 Capillary Isotachopheresis 48
- 2.2 Capillary Zone Electrophoresis 49
- 2.3 Electroosmotic Flow 50
- 3 Instrumentation 52
- 3.1 Injection 52
- 3.2 Detection 55
- 4 Applications 57
- 4.1 Peptide Separations 57
- 4.2 Protein Separations 58
- 5 References 61

II.4 Microseparation Techniques III: Gel Electrophoresis for Sample Preparation in Protein Chemistry 63

Hermann Schägger

- 1 Introduction 63
- 2 Denaturing Techniques 65
- 2.1 Commonly Used SDS-Polyacrylamide Gel Electrophoresis Techniques for Protein Separation 65
- 2.2 Blue-SDS-PAGE for Quantitative Protein Recovery from Gels 67
- 2.3 Electroelution of Proteins After Blue-SDS-PAGE 68
- 2.4 Electrobloeting of Blue and Colourless SDS Gels 69
- 2.5 Isoelectric Focusing in the Presence of Urea 69
- 3 Native Techniques 70
- 3.1 Colourless-Native-PAGE 70

- 3.2 Blue-Native-PAGE 70
- 3.3 Native Isoelectric Focusing 73
- 4 References 73

II.5 Microseparation Techniques IV: Electroblotting 75

Christoph Eckerskorn

- 1 Introduction 75
- 2 Electroblotting 76
 - 2.1 Polyacrylamide Gel Electrophoresis 76
 - 2.2 Blot Systems 76
 - 2.2.1 Tank Blotting 76
 - 2.2.2 Semidry Blotting 78
 - 2.3 Blotting Parameters 80
 - 2.3.1 The Blotting Process 80
 - 2.3.2 Transfer Buffers 81
 - 2.3.3 Addition of SDS 81
 - 2.3.4 Addition of Methanol 82
 - 2.3.5 Influence of Protein Concentration 82
- 3 Blotting Membranes 82
- 4 References 87

Section III: Amino Acid Analysis

III.1 Amino Acid Analysis 93

Roland Kellner, Helmut E. Meyer and Friedrich Lottspeich

- 1 Introduction 93
- 2 Sample Preparation 94
 - 2.1 Peptides and Proteins 94
 - 2.1.1 Enzymatic Hydrolysis 94
 - 2.1.2 Acid Hydrolysis 95
 - 2.1.3 Alkaline Hydrolysis 96
 - 2.2 Free Amino Acids 97
- 3 Derivatization 97
 - 3.1 Post-Column Derivatization 99
 - 3.1.1 Ninhydrin 99
 - 3.1.2 Orthophthaldialdehyde 99
 - 3.1.3 Fluorescamine 101
 - 3.2 Pre-Column Derivatization 101
 - 3.2.1 Phenylisothiocyanate 101

- 3.2.2 Orthophthaldialdehyde 102
- 3.2.3 Fluorenylmethyl Chloroformate 102
- 3.2.4 Dabsyl Chloride 102
- 3.2.5 Dansyl Chloride 103
- 3.2.6 Chiral Reagents 103
- 4 Data Evaluation 105
- 5 Instrumentation 106
- 6 Discussion 107
- 7 References 110

Section IV: Protein Sequence Analysis

IV.1 The Edman Degradation 117

Friedrich Lottspeich, Tony Houthaeve and Roland Kellner

- 1 The Edman Chemistry 117
- 1.1 Coupling, Cleavage and Conversion 117
- 1.2 Identification of the PTH Amino Acids 119
- 2 Instrumentation 121
- 2.1 The Liquid Phase Sequencer 122
- 2.2 The Solid Phase Sequencer 123
- 2.3 The Gas Phase Sequencer 123
- 2.4 The Pulsed Liquid Phase Sequencer 124
- 2.5 The Biphasic Column Sequencer 124
- 3 Difficulties of Amino Acid Sequence Analysis 125
- 3.1 The Sample and Sample Matrices 125
- 3.2 Difficulties with the Edman Chemistry 127
- 4 Protein Ladder Sequencing 128
- 5 State of the Art 129
- 6 References 130

IV.2 Analyzing Post translational Protein Modifications 131

Helmut E. Meyer

- 1 Introduction 131
- 2 Classification of Post-translational Modifications according to their Behaviour during Purification and Edman Degradation 132
- 2.1 Modifications: Stable During Purification and Edman Degradation 132
- 2.2 Modifications: Stable During Purification but Unstable during Edman Degradation 132
- 2.3 Modifications: Unstable During Purification and Edman Degradation 133

- 3 Examples 133
- 3.1 1-Methyl-Histidine 133
- 3.2 Glyco-Asparagine, Glyco-Threonine 134
- 3.3 Phospho-Tyrosine 135
- 3.4 N-Pyruvyl or N- α -oxo-butyric Acid 135
- 3.5 Gluco-Arginine 138
- 3.6 Farnesyl-Cysteine 139
- 3.7 Phospho-Serine 140
- 3.8 Phospho-Threonine 142
- 3.9 Screening for Phospho-Serine/Threonine Containing Peptides by HPLC/MS 143
- 3.10 Lanthionine, 3-Methyl-Lanthionine, Dehydroalanine,
Dehydro- α -aminobutyric Acid 145
- 4 References 145

Section V: Bioanalytical Mass Spectrometry

V.1 Analysis of Biopolymers by Matrix-Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry 149

Ute Bahr, Michael Karas and Franz Hillenkamp

- 1 Introduction 149
- 2 Development of MALDI 150
- 2.1 Mechanism of Matrix-assisted Laser Desorption/Ionization 150
- 3 Instrumentation 151
- 3.1 Time-of-flight (TOF) Mass Spectrometers 151
- 3.2 Laser Desorption Ion Source 153
- 3.3 Ion Detection and Data Collection 154
- 4 Applications 155
- 4.1 Sample Preparation 155
- 4.2 Molecular Weight Determination of Proteins and Glycoproteins 155
- 4.2.1 Accuracy of Mass Determination 158
- 4.2.2 Sensitivity and Mass Range 159
- 4.3 Analysis of Oligonucleotides 159
- 4.4 Analysis of Glycans and Glycoconjugates 161
- 5 Combination of MALDI with Biochemical Methods 162
- 5.1 Peptide Mapping of Digested Proteins by MALDI 162
- 5.2 Combination of MALDI and Gel Electrophoresis 162
- 5.3 Combination of MALDI with Capillary Zone Electrophoresis 163
- 6 Future Developments 163
- 6.1 Peptide Sequencing 163
- 6.2 "Surface" MALDI 164
- 7 References 164

V.2 Electrospray Mass Spectrometry 167*Jörg Metzger and Christoph Eckerskorn*

- 1 Introduction 167
- 2 Instrumentation 167
- 2.1 The Electrospray Source 168
- 2.2 The Mass Analyser 170
- 2.3 The Detector 171
- 3 The Ion Spectra 171
- 4 Coupling of Chromatographic Methods to the Mass Spectrometer 174
- 5 Off-line HPLC-MS 175
- 5.1 Sample Introduction with an Autosampler 176
- 5.2 Purity Control of Synthetic Peptides 177
- 5.3 Characterization of Synthetic Peptide Libraries 179
- 6 Structure Elucidation of Peptides and Proteins 184
- 6.1 HPLC Coupled to Mass Spectrometry 184
- 6.2 Capillary Electrophoresis Coupled to Mass Spectrometry 184
- 6.3 Microcapillary LC coupled to Mass Spectrometry 184
- 6.4 Practical Aspects 185
- 7 References 185

V.4 Sequence Analysis of Proteins and Peptides by Mass Spectrometry 189*Christiane Weigt, Helmut E. Meyer and Roland Kellner*

- 1 Introduction 189
- 2 Protein Sequencing by Mass Spectrometry 190
- 2.1 Tandem Mass Spectrometry 193
- 3 Strategy for Protein Sequencing with Electrospray Tandem Mass Spectrometry 195
- 4 Examples of Protein Sequencing Using Tandem Mass Spectrometry 197
- 4.1 Sequence Analysis of Peptides Presented to the Immune System by MHC Molecules 197
- 4.2 Partial Sequencing and Identification of a Phosphorylation Site of Recombinant Mitogen-Activated Protein Kinase p42mapk 199
- 4.3 Protein Sequence Analysis by Tandem Mass Spectrometry in Combination with Microcapillary HPLC 201
- 5 References 204

Section VI: Database Analysis

VI.1 Protein Sequences and Sequence Databases 209

Hans-Werner Mewes and David G. George

- 1 Introduction 209
- 2 Current Databases 210
- 3 Data Processing and Principles of Data Organisation 211
 - 3.1 Data 214
 - 3.2 Computer Science in the Development of Biological Databases 215
- 4 Access to Molecular Sequence Databases 215
 - 4.1 The ATLAS Multidatabase Information Retrieval System 217
 - 4.2 Searching Molecular Sequence Databases 219
- 5 Future Developments 220
- 6 References 221

VI.2 Mass Spectrometrical Data for Protein Sequence Analysis 223

Matthias Mann

- 1 Introduction 223
- 2 Program and Algorithm 224
 - 2.1 Requirements for Installing PeptideSearch 224
 - 2.2 Data Structures and Algorithm 225
 - 2.3 Performance 226
 - 2.4 Collaboration with Other Programs 226
- 3 Searching by Total Molecular Weight 227
 - 3.1 Limitations of Searching by Total Molecular Weight 228
 - 3.2 Recommendations and Prospects for Searching by Total Molecular Weight 230
- 4 Searching by the Molecular Weight of a Set of Peptides Generated by Sequence-Specific Cleavage of a Protein 230
 - 4.1 Influence of Mass Accuracy 234
 - 4.2 Influence of Target Protein Mass Range 234
 - 4.3 Influence of Minimum Number of Peptide Matches 235
 - 4.4 Influence of Partial Digestion Setting 235
 - 4.5 Choice of Enzyme 235
 - 4.6 Avoiding False Positives 236
 - 4.7 Special Searches: Time Course Digestion, Parallel Digestion and Subdigestion 237
 - 4.8 Special Searches: DNA Database Searching 237
 - 4.9 Searching Incompletely Purified Proteins and Protein Mixtures 238
 - 4.10 Limitations of Searching by Peptide Masses 238
 - 4.11 Recommendations and Prospects of Searching by Peptide Masses 238

- 5 Searching by the Molecular Weight of a Peptide and Its Partial Sequence 239
- 5.1 Searching by Partial Sequence Obtained by Edman Degradation and the Peptide Molecular Weight 239
- 5.2 Searching by an MS/MS Pattern 240
- 5.3 Matching Peptides with Sequence Errors 242
- 5.4 Matching Peptides with Post-translational Modifications 242
- 5.5 Removing Contaminating Proteins 242
- 5.6 Searching DNA Sequence Libraries 243
- 5.7 Recommendations and Prospects 243
- 6 Conclusion 244
- 7 References 245

VI.3 Software Packages for Personal Computers 247

Bernd Eisermann and Helmut E. Meyer

- 1 Introduction 247
- 2 Overview of Protein and DNA Databanks 247
- 3 What a Program for Sequence Analysis Should Do 248
- 4 Available Program Packages 249
- 4.1 On-Line Services 250
- 4.2 Program Packages for Personal Computers 251
- 5 References 259

Index 261

Section I: Overview

Microcharacterization of Proteins

Protein Characterization

The first step in the characterization of a protein is to determine its molecular weight. This can be done by using a variety of methods, including gel electrophoresis, mass spectrometry, and size exclusion chromatography. Gel electrophoresis is a technique in which a mixture of proteins is separated on the basis of their size and charge. Mass spectrometry is a technique in which the mass-to-charge ratio of a protein is determined. Size exclusion chromatography is a technique in which a protein is separated on the basis of its size. Once the molecular weight of a protein has been determined, the next step is to determine its amino acid sequence. This can be done by using a variety of methods, including Edman degradation, mass spectrometry, and DNA sequencing. Edman degradation is a technique in which the amino acid sequence of a protein is determined by sequentially removing one amino acid at a time. Mass spectrometry is a technique in which the mass-to-charge ratio of a protein is determined. DNA sequencing is a technique in which the sequence of a protein's gene is determined.

Once the amino acid sequence of a protein has been determined, the next step is to determine its three-dimensional structure. This can be done by using a variety of methods, including X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, and molecular dynamics simulation. X-ray crystallography is a technique in which the structure of a protein is determined by measuring the diffraction of X-rays by a crystal of the protein. NMR spectroscopy is a technique in which the structure of a protein is determined by measuring the magnetic properties of the protein. Molecular dynamics simulation is a technique in which the structure of a protein is determined by simulating the movement of the protein's atoms.

Genetic Aspects

The genetic aspects of protein characterization involve the study of the genes that code for a protein. This can be done by using a variety of methods, including DNA sequencing, Southern blotting, and Northern blotting. DNA sequencing is a technique in which the sequence of a protein's gene is determined. Southern blotting is a technique in which a protein's gene is detected by hybridizing it with a labeled probe. Northern blotting is a technique in which a protein's mRNA is detected by hybridizing it with a labeled probe. Once the genetic aspects of a protein have been characterized, the next step is to determine its function. This can be done by using a variety of methods, including enzyme assays, cell culture studies, and animal models. Enzyme assays are techniques in which the activity of a protein is measured. Cell culture studies are techniques in which the effect of a protein on cells is measured. Animal models are techniques in which the effect of a protein on an animal is measured.

