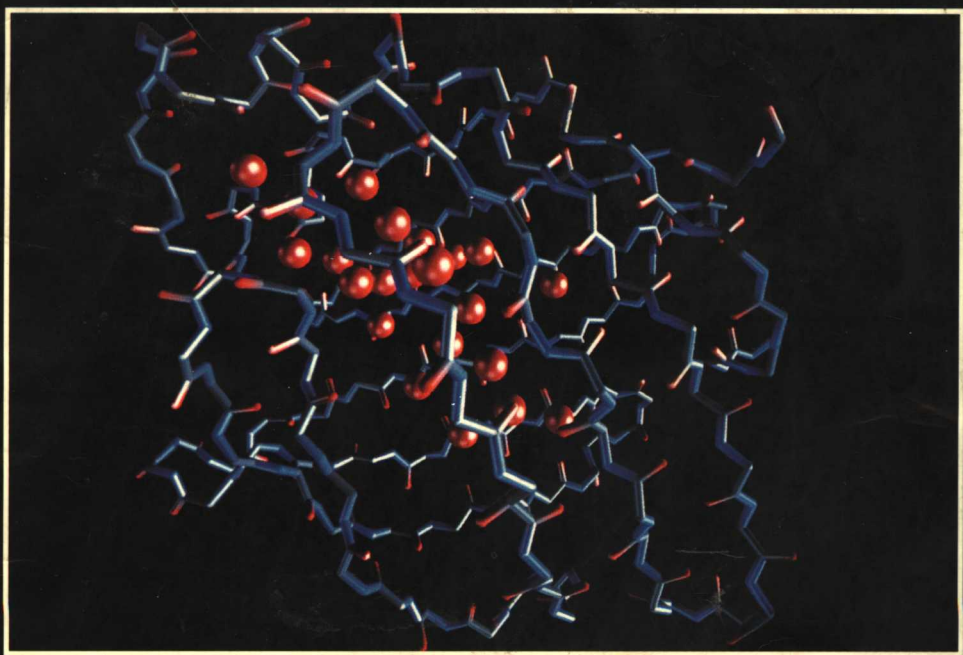


TECHNIQUES IN PROTEIN CHEMISTRY III



*Published under the Auspices of
The Protein Society*

Edited by Ruth Hogue Angeletti

TECHNIQUES IN PROTEIN CHEMISTRY III

Edited by

Ruth Hogue Angeletti

Albert Einstein College of Medicine
Bronx, New York



ACADEMIC PRESS, INC.
Harcourt Brace Jovanovich, Publishers
San Diego New York Boston
London Sydney Tokyo Toronto

Academic Press Rapid Manuscript Reproduction

This book is printed on acid-free paper. ∞

Copyright © 1992 by ACADEMIC PRESS, INC.

All Rights Reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Academic Press, Inc.
San Diego, California 92101

United Kingdom Edition published by
Academic Press Limited
24-28 Oval Road, London NW1 7DX

International Standard Book Number: 0-12-058755-6 (Hardcover)
International Standard Book Number: 0-12-058756-4 (Paperback)

PRINTED IN THE UNITED STATES OF AMERICA

92 93 94 95 96 97 EB 9 8 7 6 5 4 3 2 1

Foreword

My first graduate student learned quickly that if you want to draw a huge crowd around your poster at a meeting, don't show your latest results from an investigation of a biological problem, but present a hot new technique applicable to many problems. Advances in understanding biological systems draw the specialists, admittedly the most important people in your field; but making it possible for anyone to do something more quickly, at higher sensitivity and accuracy, and with less input of supplies and labor draws everyone.

I suppose that this is the wrong lesson for a graduate student to learn; *solving the problem* is the objective after all. But we are always pressing the limits of our assays, and each advance in sensitivity and speed opens new fields of research which were previously inaccessible. Thus, scientists will always be looking for better ways of doing things, will always cite useful technique papers more than those advancing their field of research (Oliver Lowry, where would we be without you?), and will always be interested in books reporting the latest in methodology.

This is the fourth volume resulting from the Protein Society symposia. The number of copies sold and the desire of investigators to contribute to these volumes (our editors always comment on the willingness of the authors to submit their manuscripts *on time!*) are indeed impressive. The Protein Society has always devoted a lot of attention at its meetings to new methodologies, and the Fifth Symposium in Baltimore, June 22-26, 1991, from which this book was compiled, was no exception. There were more posters than ever at the symposium, and their quality was outstanding. We are grateful for that continuing interest and will commit ourselves to fostering it in any way possible.

Special thanks are due Ruth Hogue Angeletti and her associates for editing this volume. Their diligence and the top quality work presented at the Fifth Symposium make this a most useful and informative book.

Mark Hermodson
President
The Protein Society

Preface

The pleasant surprise in editing this volume has been the enthusiastic cooperation of the members of the Protein Society. Without this support, it would have been impossible to produce a book whose ideas are still fresh and current at publication. This active participation indicates to me that the Techniques in Protein Chemistry volumes have truly become our forum for exchanging new methods and research approaches and reflect the spirited exchanges of the poster sessions.

My special appreciation is due those who made this book possible: the associate editors John Crabb, Steve Carr, Phil Andrews, and Ira Ropson; the special reviewers Al Smith and Ed Nieves; the staff of Academic Press; the presidents of the society Finn Wold and Mark Hermodson; my secretary Elyse Rizzo for her organization and attention to detail; Jim Sacchettini, who created the cover graphics; and the authors, who did the real work while tolerating my gentle, but persistent nagging. I hope each volume published becomes used and "dogeared."

Ruth Hogue Angeletti

Acknowledgments

The Protein Society acknowledges with thanks the following organizations who through their support of the Society's program goals contributed in a meaningful way to the fifth annual symposium and thus to this volume.

AAA Laboratory
Amgen, Inc.
Applied Biosystems, Inc.
AVIV Associates, Inc.
Beckman Instruments, Inc.
Boehringer Mannheim Corporation
Dionex Corporation
Hewlett-Packard
Immuno-Dynamics, Inc.
Merck, Sharp & Dohme Research Laboratories
Millipore Corporation
Molecular Dynamics, Inc.
Monsanto Co.
National Biomedical Research Foundation
PE/SCIEX
PerSeptive Biosystems
Pharmacia LKB Biotechnology
Pickering Laboratories, Inc.
Polygen Corporation
Proton Instruments, Inc.
Rainin Instrument Co., Inc.
Savant Instruments, Inc.
Vestec Corporation
Vydac, The Separations Group, Inc.

Contents

<i>Foreword</i>	xiii
<i>Preface</i>	xv
<i>Acknowledgments</i>	xvii

Section I

Protein Microsequence Methods

Protein and Peptide Recovery from PVDF Membranes	3
<i>Susan Wong, Allan Padua, and William J. Henzel</i>	
Automated C-Terminal Sequencing of Peptides	11
<i>Jerome M. Bailey, Narmada R. Shenoy, and John E. Shively</i>	
Elution and Internal Amino Acid Sequencing of PVDF-Blotted Proteins	23
<i>Kathryn L. Stone, Dean E. McNulty, Mary L. LoPresti, J. M. Crawford, Raymond DeAngelis, and Kenneth R. Williams</i>	
Evaluation of Protein Sequencing Core Facilities: Design, Characterization, and Results from a Test Sample (ABRF-91SEQ)	35
<i>Dan L. Crimmins, Gregory A. Grant, Liane M. Mende-Mueller, Ronald L. Niece, Clive Slaughter, David W. Speicher, and K. Ümit Yüksel</i>	
Optimization of Solid Phase Peptide Sequencing	45
<i>Arie Admon and David S. King</i>	
Evaluation of the Blott Cartridge for Enhanced Gas Phase Sequencing at Maximum Sensitivity	53
<i>David F. Reim, Peter Hembach, and David W. Speicher</i>	

- Reusing PVDF Electroblotted Protein Samples After N-Terminal Sequencing to Obtain Unique Internal Amino Acid Sequence 61

Cynthia L. Wadsworth, Mark W. Knuth, Laura W. Burrus, Bradley B. Olwin, and Ronald L. Niece

- Chromatographic Carbon as an Inert Sample Adsorbent for Protein Sequencing 69

Christopher Southan

- N^α-Acylaminoacyl-Peptide Hydrolase: Specificity and Use to Unblock N-Acetylated Proteins 77

Radha G. Krishna

Section II

Protein Microsequencing Workshop

- Strategies for the Isolation of Peptides from Low-Abundance Proteins for Internal Sequence Analysis 87

Ruedi Aebersold, Scott D. Patterson, and Daniel Hess

- High-Sensitivity Peptide Mapping Utilizing Reversed-Phase Microbore and Microcolumn Liquid Chromatography 97

Robert J. Moritz, Larry D. Ward, and Richard J. Simpson

- Microscale Protein Isolation and Microsequencing Workshop: An Overview 107

John E. Shively

Section III

Peptide/Protein Separation Techniques

- Micropreparative Separation of Tryptic Digests by Capillary Electrophoresis and Characterization by Protein Sequencing 113

Alan Smith, James W. Kenny, and J. I. Ohms

- Estimation of Protein Isoelectric Points by Capillary Electrophoresis Using MICRO-COAT™ 121

Michael F. Rohde, Kendall S. Stoney, and John E. Wiktorowicz

**Capillary Electrophoresis for Preparation of Peptides and Direct
Determination of Amino Acids 129**

Tomas Bergman and Hans Jörnvall

**Perfusion Chromatography: A Novel Tool for Protein Purification
and Analysis 135**

*Noubar B. Afeyan, Neal F. Gordon, Scott P. Fulton, and
Fred E. Regnier*

**Comparison of Coupling Procedures for Development of Affinity
Membranes: Optimization of the CDI Method 151**

Pamela W. Feldhoff

**Section IV
Peptide Synthesis**

**Randomization of Amino Acid Sequence: An Important
Control in Synthetic Peptide Analogue Studies of Nucleic Acid
Binding Domains 163**

*Steven G. Nadler, Janet L. Kapouch, James I. Elliott,
and Kenneth R. Williams*

**Comparative Analysis of Synthetic Peptides by Free-Solution
Capillary Electrophoresis (FSCE) and Strong-Cation Exchange
(SCX)-HPLC 171**

Dan L. Crimmins

**Immunoreactive Synthetic Peptide Epitope Mapping with Structural
Validation Using Electrospray Mass Spectrometry 183**

*William T. Moore, Jerry S. Wolinsky, Marc J.-F. Suter,
Terry B. Farmer, and Richard M. Caprioli*

TFMSA/TFA Cleavage in t-Boc Peptide Synthesis 199

Farzin Gharahdaghi, Jeffrey Mathers, and Sheenah M. Mische

**Synergy of Semisynthesis and Site-Directed Mutagenesis:
Manipulating Genes to Create Sites for Autocatalytic Protein
Fragment Religation 209**

*Carmichael J. A. Wallace, J. Guy Guillemette, Michael Smith,
and Yuko Hibiya*

State-of-the-Art Peptide Synthesis: Comparative Characterization of
a 16-mer Synthesized in 31 Different Laboratories 219

*A. Smith, J. D. Young, S. A. Carr, D. R. Marshak, L. C. Williams,
and K. R. Williams*

Section V
Amino Acid Analysis

Applications of Automatic PTC Amino Acid Analysis 233

Karen A. West and John W. Crabb

LC-Determination of D- and L-Amino Acids in Peptide Hydrolysates
by Pre-column Derivatization with a Chiral Reagent 243

Hans Brueckner, Matthias Langer, and Herbert Godel

Amino Acid Analysis of PVDF Bound Proteins 249

*Farzin Gharahdaghi, Donna Atherton, Michael DeMott,
and Sheenah M. Mische*

Collaborative Trial Analyses of ABRF-91AAA 261

*Daniel J. Strydom, George E. Tarr, Yu-Ching E. Pan,
and Raymond J. Paxton*

Section VI
Structural Analysis of Glycoproteins

Identification of O-Glycosylation Sites with a Gas Phase
Sequencer 277

John L. Abernethy, Yang Wang, Allen E. Eckhardt, and Robert L. Hill

Glycoform, Glycotype Linkage, and Branching Detail of the CD2
Adhesion Domain by Desolvation Mass Spectrometry: SFCI-MS
and ESI-MS 287

*Bruce B. Reinhold, Ellis L. Reinherz, Vernon N. Reinhold,
Maria H. Knoppers, and Michael A. Recney*

Characterization of the Glycosylation on Recombinant Human Low-Affinity Nerve Growth Factor Receptor 295

Christine A. Settineri, Iris Leung, Lawrence S. Cousens, Barbara Chapman, Frank R. Masiarz, and Alma L. Burlingame

Nonenzymic Glycation of Hemoglobin: Structural Determinants of Site Selectivity 303

Parimala Nacharaju and A. Seetharama Acharya

A Unified Approach to Glycoprotein Primary Structure Analysis: Identification, Isolation, and Characterization of Both Peptide and Pendant Carbohydrate of Glycopeptides 315

David H. Hawke, Kuo-Lian Hsi, Lynn R. Zieske, Ling Chen, and Pau-Miau Yuan

**Section VII
Protein Folding and Conformation**

Refolding of Human Recombinant Insulin-like Growth Factor II (IGF-II) *in Vitro*, Using a Solubilizing Affinity Handle 329

Göran Forsberg, Elisabet Samuelsson, Henrik Wadensten, Tomas Moks, and Maris Hartmanis

Conformational Stability of the Molten Globule of Cytochrome c: Role of Electrostatic Repulsion 337

Yuji Goto and Shin Nishikiori

Calcium-Induced Folding of Troponin-C: Formation of Homodimeric and Heterodimeric Two-Site Domains from Synthetic Peptides 347

Gary S. Shaw, Robert S. Hodges, and Brian D. Sykes

The Role of Aqueous Solvation in Protein Folding: Insights from Aqueous-Alkane Partitioning 355

Jonathan M. Keske, J. Malcolm Bruce, and P. Leslie Dutton

Molecular Characterization of an Aggregate Formed by a Bovine Growth Hormone Folding Intermediate 363

S. Russ Lehrman, Jody L. Tuls, and Henry A. Havel

- Use of Sequence Hydrophobic Moment to Analyze Membrane Interacting Domains of Butulinum, Tetanus, and Other Toxins 373

Bal Ram Singh and Xu-hai Be

- △ Fourier Transform Infrared Spectroscopic Analysis of Proteins in Terms of Detectability, Conformation, and Surface Adsorption Density 385

Bal Ram Singh, Fen-Ni Fu, and Michael P. Fuller

- Recombinant Hemoglobins for the Elucidation of a New Mechanism of Oxygen Affinity Modulation by Cl⁻ Ions 399

Clara Fronticelli

- An Improved Strategy for the Determination of the Role in Catalysis of Amino Acid Residues in Thymidylate Synthase 407

James R. Appleman and J. Ernest Villafranca

- Protein Conformational Changes in the μ s Time Region Investigated with a Laser Pulse Photolysis Technique 417

Andrew P. Billington, Norio Matsubara, Watt W. Webb, and George P. Hess

- Macromolecular Dynamics Observed by Intramolecular Energy Transfer Using Frequency-Domain Fluorometry 429

J. R. Lakowicz, J. Kuśba, I. Gryczynski, H. Szmanski, W. Wiczk, and M. L. Johnson

- Stopped-Flow Circular Dichroism and ¹⁹F NMR as Probes for the Folding of Rat Intestinal Fatty-Acid Binding Protein (IFABP) 437

Ira J. Ropson, Jeffrey I. Gordon, David P. Cistola, and Carl Frieden

Section VIII

Mass Spectrometry

- Analysis of Protein-Protein Binding with Laser Desorption Mass Spectrometry: Can Multimeric Forms Be Studied? 447

Marc J.-F. Suter, William T. Moore, Terry B. Farmer, John S. Cottrell, and Richard M. Caprioli

Applications of Liquid Chromatography–Electrospray Mass Spectrometry (LC–ES/MS) 457

*Kristine M. Swiderek, Shiuan Chen, Gottfried J. Feistner,
John E. Shively, and Terry D. Lee*

Analysis of Proteins by Mass Spectrometry 467

*Patrick R. Griffin, Karen Furer-Jonscher, Leroy E. Hood,
John R. Yates, III, Jae Schwartz, and Ian Jardine*

Characterization of PEGated Superoxide Dismutase 477

*M. M. Vestling, C. M. Murphy, C. Fenselau, J. Dedinas,
M. S. Doleman, P. B. Harrsch, R. Kutny, D. L. Ladd, and M. A. Olsen*

**N- ϵ -Acetylation Can Occur at Lysine Residues 157, 167, 171, and 180
of Recombinant Bovine Somatotropin 487**

*Gary C. Harbour, Robert L. Garlick, Stephen B. Lyle, Frank W. Crow,
Russell H. Robins, and John G. Hoogerheide*

**Plasma Desorption Mass Spectral (PDMS) Analysis of Polypeptides
Containing Some Post-translational Modifications; Scission of
Covalent Bonds 497**

Koji Takio and Koiti Titani

**Determination of the Cleavage Site of the Amyloid Precursor Protein
by Plasma Desorption Mass Spectrometry 505**

*Rong Wang, Robert J. Cotter, James F. Meschia, and Sangram S.
Sisodia*

**Large-Scale Protein Mapping Using Infrequent Cleavage Reagents,
LD TOF MS, and ES MS 515**

P. C. Andrews, M. H. Allen, M. L. Vestal, and R. W. Nelson

**Tandem Mass Spectrometric Sequencing of Proteins Isolated from 1-
and 2-Dimensional Polyacrylamide Gel Electrophoresis 525**

*Stemen C. Hall, Patrick A. Schindler, Frank R. Masiarz,
and Alma L. Burlingame*

Index 533

SECTION I

Protein Microsequence Methods

Protein and Peptide Recovery from PVDF Membranes

Susan Wong, Allan Padua and William J. Henzel

Department of Protein Chemistry, Genentech, Inc.
South San Francisco, CA 94080

I. Introduction

Electroblotting proteins from gels onto solid supports is a widely utilized technique for the microisolation of proteins. Several electroblotting membranes have been developed that allow direct sequence analysis (1-3). Polyvinylidene difluoride (PVDF) is the most frequently utilized support for sequence analysis of electroblotted proteins (4). A limitation of this technique is that N-terminal blocked proteins must be chemically cleaved or proteolytically digested to generate internal peptide fragments which can then be sequenced. *In situ* digestions on PVDF (5-8) and nitrocellulose membranes (9) have been described; however, these methods only result in partial digestion of the membrane-bound proteins. Methods that directly extract protein from PVDF have been developed (10,11), but are based on solutions containing detergents which can limit subsequent proteolytic digestions or interfere with HPLC separations. We have developed a simple extraction procedure using the solvent dimethyl sulfoxide (DMSO) that allows recovery of intact proteins from PVDF membranes. The DMSO can be easily removed, allowing the extract to be analyzed by protein sequencing, amino acid analysis, or by other biochemical analysis.

The extracted protein can be cleaved or digested and the resulting fragments can either be separated by HPLC or by tricine SDS-gels (12) followed by a second round of electroblotting. An additional advantage of this technique is that the protein may be directly digested in the extraction solvent after addition of an appropriate buffer.

II. Materials and Methods

Materials. Proteins were purchased from Sigma, with the exception of human growth hormone (hGH) which was obtained from Genentech, Inc. Lys-C was from Wako chemicals, and cyanogen bromide was from Pierce. Dimethyl sulfoxide (spectrometric grade) was from Aldrich. PVDF (Immobilon-P) membranes were obtained from Millipore and ProBlott membranes were from Applied Biosystems.

Electroblotting and elution. SDS-PAGE was done as described by Laemmli (13) using a BioRad minigel apparatus. Tricine minigels were obtained from Novex. Electroblotting was done in a BioRad transfer tank using 10 mM 3-[cyclohexylamino]-1-propanesulfonic acid, 20% methanol, pH 11.0 for 1 h at 250 mA (4). Coomassie stained bands from electroblotted proteins were extracted with 200 μ l DMSO in a micro Eppendorf tube on a VWR vortexer-2 using a medium setting for 2 h. The DMSO was removed and another 200 μ l of DMSO was added. The extraction was continued for an additional 2 h. The extracts were then combined and used for further experiments. Extractions were also performed using a single addition of 200 μ l of DMSO, vortexed for 17h.

Fragmentation. Extracted proteins were dried in a Speed-Vac to remove DMSO and were cleaved with cyanogen bromide in 0.1 N HCl at 45°C for 3 h (14). Blots were prewetted with 5 μ l of methanol prior to the addition of 0.1N HCl and cyanogen bromide.

Protease digestions were performed on electroblot extracts after partial removal of DMSO by Speed-Vac evaporation. Ammonium bicarbonate (0.1 M) was added to the residual DMSO (20-50 μ l) resulting in a final solution of 200-500 μ l of 10% DMSO. Digestion with Lys-C was carried out at 37°C for 17 h. with an enzyme to substrate ratio of 1:20 (w/w).

Electroblots were first treated with a modified methanol-chloroform precipitation (15) prior to DMSO elution in order to remove Coomassie blue and HPLC artifact peaks. Blots (wet) were placed in 100 μ l of water in an Eppendorf tube and 400 μ l of methanol was added. The solution was vortexed for 2 min. and 100 μ l of chloroform was added. The solution was vortexed again for 2 min. and the liquid was removed. The membrane was then extracted with DMSO. This method effectively removed the Coomassie blue and minimized artifact peaks in the HPLC chromatograms.

Peptide separation. Peptides were purified by reversed-phase HPLC using a Synchropak 4000 Å C4 column (2 x 100 mm) on a Hewlett-Packard 1090 M liquid chromatograph equipped with diode array detector. Peaks were detected at 214 nm after elution with a linear gradient of 0-70% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 200 μ l per minute.

Protein sequencing. Automated Edman degradation was performed with Applied Biosystems models 470A and 473A protein sequencers equipped with on-line PTH analyzers. Sequence interpretation was performed on a VAX 8750 as described (16).

Amino acid analysis. Extracted proteins were dried in a Speed-Vac to completely remove DMSO. Residual DMSO can result in the oxidation of some amino acids including methionine and cysteine. Blots were prewetted with 5 μ l of methanol prior to hydrolysis. Proteins were hydrolyzed in the gas phase for 24 h with 300 μ l 6N HCl containing 0.1% phenol at 110°C under vacuum using a Millipore Picotag workstation. Hydrolyzed blots were extracted twice with 100 μ l of 6 N HCl. The extracts were dried and reconstituted with Beckman sample buffer and hydrolysates were analyzed on a Beckman Model 6300 amino acid analyzer.