CONTENTS

A. METHODS OF POLYPEPTIDE PURIFICATION AND CHARACTERIZATION

I. Chromatographic Methods of Polypeptide Purification

Use of Short HPLC Column For The Concentration, Separation and Recovery of Subnanomole Amounts of Protein and Polypeptides For Microsequence Analysis
Richard J. Simpson, Boris Grego, Michael R. Rubira, Lindsay G. Sparrow and Edouard C. Nice

Practical and Theoretical Aspects of Microbore HPLC of Proteins, Peptides and Amino Acid Derivatives
Kenneth J. Wilson, David R. Dupont, Pau M. Yuan, Michael W. Hunkapiller and Timothy D. Schlabach

Microisolation and Sequence Analysis of Human Epidermal Growth Factor
Petro E. Petrides, Peter Bohlen and Frederick S. Esch

Complete Amino Acid Sequence Determination of Rat Epidermal Growth Factor: Characterization of a Truncated Form With Full In Vitro Biological Activity
Edouard C. Nice, John A. Smith, Robert L. Moritz, Michael J. O'Hare, Phillip S. Rudland, John R. Morrison, Christopher J. Lloyd, Boris Grego, Antony W. Burgess and Richard J. Simpson

Use of HPLC Comparative Peptide Mapping in Structure/Function Studies

Pre-column Derivatization of Peptides With Phenlisothiocynate (PITC)
Simon Lemaire and Serge Nolet

High-Performance Liquid Chromatography as a Means of Characterizing Isoforms of Steroid Hormone Receptor Proteins
J.L. Wittliff, N.A. Shahabi, S.M. Hyder, L.A. van der Walt, L. Myatt, D.M. Boyle and Y.-J. He
Large Scale Purification of a Human Hepatoma Derived Endothelial Cell Growth Factor
Robert C. Sullivan, John A. Smith, Ricky Nelson, Yuen W. Shing and Michael Klagsburn

Very Rapid Microanalysis of IgG in Ascites Fluids By HPLC Using a Novel Anion-Exchange Column
David L. Burke and J. Keith Duncan

Application of Immunoaffinity Chromatography to the Purification of Polypeptides for Microsequence Analysis
James J. L'italien

II. Electrophoretic Methods of Polypeptide Purification

A Novel Approach to Isolation of Proteins for Microsequences Analysis: Electroblotting onto Activated Glass
Ruedi Aebersold, David B. Teplow, Leroy E. Hood and Stephen B.H. Kent

Blotting of Protein-Detergent Complexes on Glass and Glass Coated with Polybases: A Comparative Study. Acid Hydrolysis and Gas-Phase Sequencing of Gel-Separated Proteins Immobilized on Polybrene Glass Fiber
Joel Vandekerckhove

Two-Dimensional Gel Electrophoresis of Polypeptides
Daryll B. DeWald, Lonnie D. Adams and James D. Pearson

III. Chemical Modification of Polypeptides

Carboxymethylated Hemoglobin as a Structural Analogue For Carboxamin Hemoglobin
Wendy J. Fanti, Alberto Di Donato, Arthur Arnone and James M. Manning

Gossypol: Interaction with Ribonuclease A
Hiroshi Ueno, Samuel S. Koide, James M. Manning and Sheldon J. Segal

Studies on Nonenzymic Glycosylation of Peptides in a Simple Model System
Nobuhiro Mori and James M. Manning

Reaction of Glyceraldehyde (Aldotriose) with Proteins is a Prototype of Nonenzymic Glycation: Protein Cross-linking as a Consequence of In Vitro Non-Enzymic Glycation
A. Seetharama Acharya
Reversible Dihydroxypropylation of Amino Groups in Proteins: Application in Primary Structural Studies of Streptococcal M-Proteins

Belur N. Manjula, Vincent A. Fischetti, Thomas Fairwell and A. Seetharama Acharya

Enzymatic Carboxylmethylation and Non-Enzymatic Demethylation at L-Isoaspartyl Residues: Possible Applications in Peptide Chemistry

E. David Murray, Jr.

IV. Amino Acid Analysis of Polypeptides

A Practical Guide to the General Application of PTC-Amino Acid Analysis

Robert L. Heinrikson, Rene Mora and John M. Maraganore

High Performance Liquid Chromatography of Phenylthiodantoin and Phenylthiocarbamyl Amino Acids

Barry N. Jones, Angelo P. Consalvo, Lisa LeSueuer, Susan Lovato, Stanley D. Young, James A. Koehn and James P. Gilligan

Amino Acid Analysis Using Pre-Column Derivatization with Phenylisothiocyanate: Matrix Effects and Tryptophan Analysis

Steven A. Cohen, Thomas L. Tarvin and Brian A. Bidlingmeyer

An Improved Reverse Phase HPLC Separation and Post-Column Detection of Amino Acids

Stephen Gruber, Noel M. Meltzer, Stanley Stein and Guillermo I. Tous

HPLC Assay of Phosphoamino Acids by Fluorescence Detection

Michael J. Watson, Joan R. Kanter and Laurence L. Brunton

Amino Acid Analysis by Reverse-Phase High Performance Liquid Chromatography: Separation of Phenylthiocarbamyl Amino Acids by Spherisorb Octadecysilane Columns

Chao-yuh Yang, Felix I. Sepulveda, Tseming Yang and Wei-Yong Huang

V. Mass Spectrometry of Polypeptides

Fast Atom Bombardment Mass Spectrometry: Application to Peptide Structural Analysis

Blair A. Fraser
Routine Analysis of Low-Picomole-Level Phenylthiohydantoins by HPLC Using a Diisopropylethylamine-Acetate/THF Buffer and Acetonitrile Gradient .............................................. 395
Carl J. March and Thomas P. Hopp

Identification of Side-Chain Protected L-Phenylthiohydantoins on Cyano HPLC Columns: An Application to Gas-Phase Microsequencing of Peptides Synthesized on Solid-Phase Supports ...................................... 403
Daniel J. McCormick, Benjamin J. Madden and Robert J. Ryan

Separation of Amino Acid Phenylthiohydantoin Derivatives by High Pressure Liquid Chromatography ............................................................. 415
Joseph L. Meuth and J. Lawrence Fox

VIII. Computer Analysis of Protein Sequence Data

Hydrophobic Moments as Tools for Analysis of Protein Sequences and Structures ......................................................... 425
David Eisenberg, William Wilcox and Steven Eshita

Identification of Protein Surfaces and Interaction Sites by Hydrophilicity Analysis ......................................................... 437
Thomas P. Hopp

The Protein Identification Resource (PIR): An On-Line Computer System for the Characterization of Proteins Based on Comparisons with Previously Characterized Protein Sequences ........................................ 445
David G. George, Winona C. Barker and Lois T. Hunt

Computer Analysis of Protein Sequencing Data ................................................. 455
Norman Froelich, Lynn C. Williams, John T. Casagrande and Minnie McMillian

IX. Miscellaneous Methods of Polypeptide Characterization

Simultaneous Multiple Peptide Synthesis: The Rapid Preparation of Large Numbers of Peptides ................................................. 463
Richard A. Houghten, Sarah R. Hoffman, J.M. Ostresh Mairead K. Bray, Nicole Frizzell, Suzanne M. Pratt and John Sitraik

Mapping Functional Domains of Human Platelet Thrombospondin with Electroblotting and High Sensitivity Sequencing ................................................. 471
Gregory A. Grant, Vishva M. Dixit, Nancy J. Galvin, Karen M. O'Rourke, Samuel A. Santoro and William A. Frazier

Immunoprotection - A Novel Approach for Mapping Epitopes on an Antigen ................................................. 479
Betsy J. Bricker, Robert R. Wagner and Jay W. Fox
Micro Method for the Detection of Heparin-Binding Proteins and Peptides
Nobuyoshi Hirose, Lilian Socorro, Richard L. Jackson, and Alan D. Cardin

Rapid and Sensitive Determination of Protein Disulfide Bonds
Hsieng S. Lu, Michael L. Klein, Richard R. Everett and Por Hsiung Lai

Simulation of Protein Hydrodynamic Changes Observed by Urea Gradient Gel Electrophoresis
William Shalongo and Earle Stellwagen

B. ANALYSIS OF POLYPEPTIDE STRUCTURE AND FUNCTION

X. Site-Directected Mutagenesis

Site-Specific Mutations in Dihydrofolate Reductase at the Dihydrofolate Binding Site
Ruth J. Mayer, Jin-Tann Chen, Kazunari Taira, and Stephen J. Benkovic

Site Saturation Mutagenesis of Active Site Residues of \( \beta \)-Lactamase
Steve C. Schultz, Steven S. Carroll and John H. Richards

Enzyme Thermostability and its Enhancement by Genetic Engineering
Tim J. Ahern and Alexander M. Klibanov

Expression of Human C5a in E. coli
Chen-Chen Kan, Yoshihiro Fukuoka, Tony E. Hugli and Georg H. Fey

XI. Active Site Studies

Use of Trinitrobenzene Sulfonate to Determine the pK\(_a\) Values of Two Active-Site Lysines of Ribulose-bisphosphate Carboxylase/Oxygenase
Fred C. Hartman, Sylvia Milanez and Eva J. Lee

Advances in Affinity Labeling of Purine Nucleotide Sites in Dehydrogenases
Roberta F. Colman
Escherichia Coli ADPglucose Synthetase Substrate-Inhibitor Binding Sites Studied by Site(s) Directed Chemical Modification and Mutant Enzyme Characterization

Young Moo Lee, Charles E. Larsen and Jack Preiss

Active Site and Other Sequence Data from Torpedo Californica Acetylcholinesterase

Kathleen MacPhee-Quigley, Thomas S. Vedvick, Palmer Taylor and Susan S. Taylor

Acetylcholine Receptor α-Bungarotoxin Interactions Studied by Chemical Modification, Enzyme Digestion and HPLC

Allan L. Bieber, Jose Carlos Garcia-Borron, and Marino Martinez-Carrion

Imidoesters and the Mechanism of Liver Alcohol Dehydrogenase

Bryce C. Plapp

Investigation of Gramicidin Channel Function by Single Amino Acid Replacement Using Non-Genetic Code Amino Acids

Roger E. Koepppe II and Olaf S. Andersen

 XII. Domain and Topographical Studies

Identification of Functional Domains of the Inhibitor Protein of cAMP-Dependent Protein Kinase

Heung-chin Cheng, Bruce E. Kemp, Alan J. Smith, Richard B. Pearson, Scott M. Van Pattern, Lufti Misconi and Donal A. Walsh

Atrial Natriuretic Factor Receptor in Adrenal Plamsa Membrane: Identification by Photo-Affinity Labeling

Kunio S. Misono

Structural Analysis of a 29/38kDa Heparin-Binding Domain of Fibronectin: Evidence That Two Different Subunits of Human Plasmα Fibronectin Arise by Alternative mRNA Splicing

Hema Pande, Jimmy Calaycay, Terry D. Lee, Annalisa Siri, Luciano Zardi and John E. Shively

Structural and Functional Studies on the Purified Insulin Receptor

Yoko Fujita-Yamaguchi

Localization and Interaction of Functional Sites on Antithrombin III. Use of an Anti-Hapten Antibody as a Structural Probe

Cynthia B. Peterson and Michael N. Blackburn
XIII. Characterization of Proteases

Properties of the Hydrolase that Catalyzes Removal of the Blocked NH₂-Terminal Amino Acid Residues From Polypeptides

Wanda M. Jones, Lois R. Manning, and James M. Manning

Kinetic Product Analysis of Aspartyl Proteinases Utilizing New Synthetic Substrates and Reversed Phase HPLC

Ben M. Dunn, Melba Jimenez, Jeff Weidner, Michael Pennington, Mark Carter and Benne Parten

The Esterase-Like Activity of Covalently Bound C3

Yeldur P. Venkatesh and R.P. Levine

XIV. Identification of Sites of Post-Translational Modification

Structural Analysis of Carcinoembryonic Antigen (CEA) and a Related Tumor-Associated Antigen (TEX)

Raymond J. Paxton and John E. Shively

Determination of the Location of NG,N¹Dimeethylarginine in a Glycine-Rich Region of Nuclear Protein C23

Mark O. J. Olson, Tamba S. Dumbar, S.V.V. Rao and Michael O. Wallace

Structural Characterization of a Murine Lymphocyte Homing Receptor Suggests a Ubiquitinated Branched-Chain Glycoprotein

Mark Siegelman, Martha Bond and Irving L. Weissman

XV. New Sequences

Human C3b/C4b Receptor (CR1): Isolation, Protein Sequence Analysis, and Cloning of a Partial cDNA From Human Tonsil

John A. Smith, Winnie W. Wong, Lloyd B. Klickstein, John Weis and Douglas T. Fearon

HTLV-III/LAV Particle-Associated Proteins: I. Virus Purification, Inactivation and Basic Characterization

Michael Phelan, J. Willard Hall, Martha Wells, Michele Kowalski, Luba Vujcic, Gerald Quinlan, Jr., and Jay Epstein

Structure and Function of Fish Antifreeze Polypeptides

Choy L. Hew and Peter L. Davies
Sequence of a Glycine-Rich Protein From Lizard Claw: Unusual Dilute Acid and Heptafluorobutyric Acid Cleavages
Adam S. Inglis, J. Morton Gillespie, Charles M. Roxburgh, Lois A. Whittaker and Franca Casagranda

Primary Structure Studies on Serine Hydroxymethyltransferase
Donatella Barra, Filippo Martini, Sebastiana Angelaccio, Stefano Pascarella, Francesco Bossa and LaVerne Schirch

The Proteins in Avian Eggs: A Test of Protein Separation Methods Based on HPLC
R.W. Burley and J.F. Back

Amino Terminal Sequence of 7B2: A Novel, Human Pituitary/Brain Polypeptide Present in Gonadotrophs and Released by LHRH
R. Leduc, N.G. Seidah, S. Benjannet, J.S.D. Chan, M. Marcinkiewicz, C. Lazure and M. Chrétien

C. SUMMARY OF SYMPOSIUM WORKSHOP

The Symposium Test Peptide (STP): Synthesis and Characterization of a Model Peptide
J. Rivier, R. Galyean, W. Woo, D. Karr, T. Richmond and J. Spiess

Workshop in Microanalytical Methods - Experiences With an Unknown Synthetic Peptide
James J. L'Italien and Joachim Spiess

CONTRIBUTORS

INDEX