THE PEPTIDES

Analysis, Synthesis, Biology

EDITED BY

SIDNEY UDENFRIEND
JOHANNES MEIENHOFER

Volume 7

Conformation in Biology and Drug Design

EDITED BY

VICTOR J. HRUBY

The Peptides

Analysis, Synthesis, Biology

Edited by

SIDNEY UDENFRIEND
Roche Institute of Molecular Biology
Nutley, New Jersey

JOHANNES MEIENHOFER
Hoffmann-La Roche Inc.
Nutley, New Jersey

VOLUME 7 Conformation in Biology and Drug Design

Edited by

VICTOR J. HRUBY
Department of Chemistry
University of Arizona
Tucson, Arizona

1985

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

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS INC. (LONDON) LTD.
24-28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOGING-IN-PUBLICATION DATA

Main entry under title:

The Peptides : analysis, synthesis, biology.

Vol. edited by Sidney Udenfriend, Johannes Meienhofer.
Includes bibliographies and indexes.
Contents: v. 1. Major methods of peptide bond formation.—v. 2 Special methods in peptide synthesis, part A.—[etc.]—v. 7. Conformation in biology and drug design.
1. Peptides—Collected works. I. Gross, Erhard. II. Meienhofer, Johannes. [DNLM: 1. Peptides. QU 68 P424]
QP552.P4P47 574.1'92456 78-31958
ISBN 0-12-304207-0 (v. 7)

PRINTED IN THE UNITED STATES OF AMERICA

85 86 87 88 9 8 7 6 5 4 3 2 1

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

Wolfgang Bermel (437), Institute of Organic Chemistry, J. W. Goethe University, D-6000 Frankfurt-50, Federal Republic of Germany

*Michael Blumenstein** (355), Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111

Lila M. Gierasch (405), Department of Chemistry, University of Delaware, Newark, Delaware 19716

Jerry D. Glickson (301), Department of Radiology, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205

A. T. Hagler (213), Agouron Institute, La Jolla, California 92037

Victor J. Hruby (1), Department of Chemistry, University of Arizona, Tucson, Arizona 85721

Horst Kessler (437), Institute of Organic Chemistry, J. W. Goethe University, D-6000 Frankfurt-50, Federal Republic of Germany

Robert E. Lenkinski (301), Department of Chemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Arndt Müller (437), Institute of Organic Chemistry, J. W. Goethe University, D-6000 Frankfurt-50, Federal Republic of Germany

Stanley J. Opella (405), Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Karl-Heinz Pook (437), Analytical Research, Boehringer Ingelheim KG, D-6507 Ingelheim am Rhein, Federal Republic of Germany

Peter W. Schiller (115), Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7

Robert W. Woody (15), Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523

S. Scott Zimmerman (165), Department of Chemistry, Brigham Young University, Provo, Utah 84602

* *Present address:* Department of Chemical Research, McNiel Pharmaceutical, Spring House, Pennsylvania 19477.

Preface

The Peptides is an open-ended treatise providing comprehensive and critical reviews of important developments in all areas of peptide research, including analysis, synthesis, and biology. These reviews are intended to provide critical reference material for the specialist, a guide for the novice, and a forum for the wide variety of investigators concerned with peptides and proteins.

Volume 7 is the second volume in this treatise to be devoted to the analysis of peptides, but the first to emphasize exclusively the use of *physical methods* in *peptide conformational analysis*. The conformational properties of peptides and the relationships of these conformational properties to biological properties are emphasized. I believe this volume comes at a particularly good time since there is a growing awareness of the importance of peptide conformation, dynamics, and topology as major determinants in the biological properties of these compounds. Developments over the past decade increasingly emphasize the importance of peptides as the central modulators of an ever widening variety of biological functions, including classical endocrine hormonal effects such as glucose storage and release, fat metabolism, reproduction, kidney function, cardiovascular function, gut motility, digestion, and many of the wide variety of central nervous system modulations such as analgesia, learning, memory, hunger, thermal regulation, thirst, and pain. In addition, peptides serve critical functions as ionophores, modulators of the immune system, effectors of membrane function, antibiotics, modulators of enzyme action as both agonists and antagonists, and modulators of gene expression. The remarkable potency and specificity of peptides for the above and numerous other biological functions make it clear to this observer that natural peptides and, especially, peptide analogues will be among the major drugs of the future. The ability of the modern chemist and biologist to design, synthesize, and modulate peptide structures as specific tools for fundamental research, and as drugs for specific diseases, will depend on the development of an understanding of the conforma-

tional and topological properties of peptides in relation to their biological activities.

In the chapters of this volume the authors have provided critical background material and specific examples of the uses and limitations of many of the most important physical methods needed to determine the conformational properties of peptides. Extensive development of the theoretical backgrounds of these methods is avoided, but sufficient background is provided so as not to sacrifice scientific rigor. Emphasis is placed on examples from the literature that illustrate the application of these methods to peptides of biological interest. The subtitle for this volume, *Conformation in Biology and Drug Design*, reflects the intent of this volume to provide source material for those in the field who need to develop insight into peptide conformation as it is related to biological activities, and to design peptides as potential drugs. I hope that this volume will stimulate increased use of conformational considerations in research in peptide chemistry, biology, pharmacology, and medicine.

In the first chapter, I provide a brief historical overview of the ways in which determinations of peptide conformation have been used to understand peptide bioactivity, the chemical–physical considerations needed to develop conformational–biological activity relationships for peptides, especially peptide hormones and neurotransmitters, and the emerging developments that appear most likely to provide deeper insight into the fundamental question of the relationships of peptide structure to biological function.

In Chapter 2, Robert W. Woody provides a comprehensive review of the use of circular dichroism (CD) spectroscopy to examine the conformational properties of peptides in solution. Particular attention is given to the use of CD spectroscopy to identify and distinguish a variety of secondary structural (conformational) properties often found in peptides, including α-helix, β-sheet, and reverse-turn conformations. The effects of structural and solvent purturbations on these conformational features are discussed, and in addition, examination of disulfide conformation and chirality (the quadrant rule) is provided. Specific examples of both linear and cyclic peptides are provided.

The uses of fluorescence spectroscopy to examine the special relationships of aromatic side-chain groups to one another, and its relationship to overall conformation and topology, are discussed by Peter W. Schiller in Chapter 3. Also examined is the use of peptide fluorescence studies to examine peptide–macromolecular interactions.

In Chapter 4, S. Scott Zimmerman discusses the use of various theoretical methods to calculate the conformations of peptides. A step-by-step

discussion of the methodologies used in such studies and a number of practical examples are provided.

The use of theoretical computer simulation of peptide conformational and dynamic properties has become of central importance for examining the conformational space and dynamic properties available to a peptide, and this approach is particularly useful for drug design. In Chapter 5, A. T. Hagler outlines the methods used to stimulate peptide conformations and dynamics, and provides several examples, with stereopictures, of the application of these methods to biologically active peptides.

Undoubtedly the most powerful method for examining the conformations of peptides in solution (and increasingly, the solid state) and the interaction of peptides with macromolecules is nuclear magnetic resonance (nmr) spectroscopy. The last four chapters in this volume examine various aspects of the use of nmr in peptide conformational analysis.

In Chapter 6, Robert E. Lenkinski and Jerry D. Glickson examine the use of paramagnetic ions as nmr probes of peptide conformation in solution. The development of the theory and its application to both peptide hormones and enzymes should make this approach accessible to large numbers of chemists and biologists working on peptide hormones and enzyme substrates (particularly inhibitors).

Information transfer for peptide hormones, neurotransmitters, antigens, etc. generally involves the interactions of these peptides with biological macromolecules. An understanding of the conformational and the dynamic properties of the peptide in relation to such an interaction is critical to understanding the biological properties of these compounds. In Chapter 7, Michael Blumenstein reviews the nmr methods and the peptide probes (specifically labeled hormones and proteins) that make these studies possible, and what has been learned thus far and what can be learned in the future using such methods. Several examples of peptide–protein, peptide–lipid, and peptide–nucleic acid interactions are reviewed.

Recent developments in nmr theory and instrumentation have made it possible to examine structural and conformational properties of peptides in the solid state. In Chapter 8, Stanley J. Opella and Lila M. Gierasch review the methods that make such studies possible, and then provide several examples of the application of these methods to peptides in the solid state.

In Chapter 9, Horst Kessler, Wolfgang Bermel, Arndt Müller, and Karl-Heinz Pook discuss significant recent developments in nmr spectroscopy, especially the applications of two-dimensional nmr techniques and double-quantum nmr spectroscopy, which now make it possible to determine the structural, conformational, and dynamic properties of peptides

in detail. The application of these methods to peptides is given along with the general strategy that should be followed for obtaining maximal information from such studies. These methods promise to revolutionize the studies of peptide conformations and provide new insights into peptide conformational–biological activity relationships.

I wish to thank the authors for their efforts in preparing these chapters, and especially those who finished as scheduled and their patience in waiting for the others. I wish to thank Lourdes Gallegos and Rita Little, who provided significant secretarial help in preparation of my chapter, the Nomenclature and Abbreviations section, and the Index. Finally I want to express my gratitude to the staff of Academic Press for their prompt production of the book.

Victor J. Hruby

Nomenclature and Abbreviations*

Abbreviations

A	adenylic acid
AII	angiotensin II
Abu	α-aminobutyric acid
AC	alternating current
Aca	aminocaproic acid
AcOH, HOAc	acetic acid
ACTH	adrenocorticotropic hormone
Aib	α-aminoisobutyric acid or 2-Me-Ala
α-MSH	α-melanotropin (α-melanocyte stimulating hormone)
AMP	adenosine monophosphate
Ar	aromatic amino acid
Asu	2-aminosuberic acid
ATP	adenosine triphosphate
AVP	arginine vasopressin
Aze	azetidine-2-carboxylic acid
β-PPP	β-phenylpropionyl-L-phenylalanine
BK	bradykinin
BM	Bohr magneton
Boc, *t*Boc	*tert*-butyloxycarbonyl

* All symbols and abbreviations used in this volume are listed except the three-letter symbols of the common amino acids. For peptide size nomenclature, abbreviation policy, and oxazolone designation, see Volumes 1–3. The one-letter symbols for amino acids are as follows:

A alanine	G glycine	M methionine	S serine
C cysteine	H histidine	N asparagine	T threonine
D aspartic acid	I isoleucine	P proline	V valine
E glutamic acid	K lysine	Q glutamine	W tryptophan
F phenylalanine	L leucine	R arginine	Y tyrosine

BPTI	bovine pancreatic trypsin inhibitor
*t*Bu	*tert*-butyl
Bzl	benzyl
C	cytidylic acid
^{13}C	carbon-13
CAMD	computer-aided molecular design
CD	circular dichroism
Cha	cyclohexylalanine
CIDNP	chemically induced dynamic nuclear polarization
CIDS	circular intensity differential scattering
CNS	central nervous system
COLOC	correlation via long-range coupling
COSY	two-dimensional correlated spectroscopy
CP	cross-peak
CRF	corticotropin-releasing factor
CTBr	hexadecyl(cetyl)trimethylammonium bromide
DBM	Debye–Bohr magneton
DC	direct current
DEAE	diethylaminoethyl
δ	chemical shift (nmr)
DKP	diketopiperazine
DML	dimyristoyl lecithin
DMPC	dimyristoylphosphatidylcholine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
Dnp	dinitrophenyl
Dns, DNS	dansyl (5-dimethylamino-1-naphthalenesulfonyl)
DP	diagonal peak
DPBS	4-(diethylamino)phenylazobenzene-4-sulfonyl
DQF	double-quantum filter
ECEPP	empirical conformation energy program for peptides
EHT	extended Hückel method
ENK	enkephalin
EOM	electro-optic modulator
epr	electron paramagnetic spin resonance
^{19}F	fluorine-19
FID	free induction decay

Fmoc	9-fluorenylmethyloxycarbonyl
For	formyl
FT	Fourier transform
g	electronic *g* value
G	guanylic acid
G6PD	glucose-6-phosphate dehydrogenase
GTP	guanosine triphosphate
^{1}H	proton
^{2}H	deuteron
HOAc, AcOH	acetic acid
HPLC	high-pressure liquid chromatography
HyIv	hydroxyisovaleric acid
Hyp	4-hydroxyproline
Hz	hertz
im	imidazole
*i*Pr	isopropyl
ir	infrared
Iva	isovaleric acid
J	coupling constant
Lac	lactic acid
lcp	left circularly polarized light
LHRH	luteinizing hormone–releasing hormone, gonadoliberin (luliberin)
LVP	lysine vasopressin
M	negative torsional angle
Me	methyl
Me-Ala	*N*-methylalanine
MeOH	methanol
mRNA	messenger ribonucleic acid
α-MSH	α-melanotropin (α-melanocyte stimulating hormone)
^{14}N	nitrogen-14
^{15}N	nitrogen-15
Nle	norleucine

NMA	*N*-methylacetamide
nmr	nuclear magnetic resonance
NO_2	nitro
NOE	nuclear Overhauser enhancement
NOESY	nuclear Overhauser and exchange spectroscopy
NP	neurophysin
NPS	nitrophenylsulfonyl
Nva	norvaline
obs	observed
ODS	octadecyl (reversed-phase HPLC column)
ORD	optical rotatory dispersion
P	positive torsional angle
P_M	mole fraction of bound ligand
PC	partition chromatography
PEM	photoelastic modulator
Pen	half-penicillamine (β,β-dimethylcysteine)
Ph	phenyl
Phol	phenylalaninol
pmr	proton magnetic resonance
pNA	*p*-nitroaniline
ppm	part(s) per million
β-PPP	β-phenylpropionyl-L-phenylalanine
*i*Pr	isopropyl
Pyr	pyridine
q	coordination number
rcp	right circularly polarized light
RNase	ribonuclease
RP	reversed phase
S_1	first excited singlet state
Sar	sarcosine
SCF	self-consistent field
SDS	sodium dodecylsulfate
SECSY	spin–echo correlated spectroscopy
S/N	signal-to-noise ratio
Sta	statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid
T_1	spin lattice (longitudinal) relaxation time
T_2	transverse relaxation time

*t*Boc, Boc	*tert*-butyloxycarbonyl
*t*Bu	*tert*-butyl
TOCSY	total correlation spectroscopy
TOE	truncated driven nuclear Overhauser enhancement
TRNOE	transfer of nuclear Overhauser effect
VIP	vasoactive intestinal polypeptide
VP	vasopressin
Z	benzyloxycarbonyl

Contents