volume 3

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

edited by
Boris Weinstein

CHEMISTRY AND BIOCHEMISTRY OF AMINO ACIDS, PEPTIDES, AND PROTEINS

A Survey of Recent Developments

◄ *Volume 3* ►

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ABOUT THE SERIES

The amide bond is one of the less reactive organic functional groups, yet it serves as the cornerstone for the building of the many peptides and proteins found in living systems. The evolving science of molecular biology has served to stress again that the chemistry and biochemistry of amino acids, peptides, and proteins is interwoven into a complex pattern, which on closer examination is found to be dependent on a host of secondary factors associated with individual compounds. There has been a need for a new review series in this area, especially if the interrelationships between the various disciplines are to be discussed in a detailed fashion. In an ideal sense, each volume should contain some chapters on recent developments and applications of established techniques, whereas others might describe the background and problems for topics still under investigation. Too, the subjects encompassed here do permit a variety of treatments without undue duplication or specialization.

One need not remind the reader of the many life processes that are dependent upon specific amino acid, hormone, and enzyme systems. Each functions in a very unique fashion, yet, in the end, they must involve the reactions of fundamental organic chemistry. Sometimes this point is overlooked, and it will be restated in greater detail through the series. To balance the cale, the brief comment is made here that new protecting, labeling, and coupling agents are always desirable, but these must be put to the test by the synthesis or degradation of actual compounds, for which practical use exists in Nature.

It is anticipated that these volumes can be useful both to the specialist and nonspecialist, and may provide a reference point to those who may do research in a broad region, or to the active worker in a small field. Most importantly, these volumes can serve the general purpose of presenting various points of view on the amide bond to interested observers, who, at present, are unknown to one another.

Boris Weinstein

Seattle, Washington
December, 1970

PREFACE

Due to unexpected delays in the production of volume two in this series, volume three follows rather quickly after its predecessor. However, future ones should come at more regular intervals.

The first chapter by Victor J. Hruby is a very complete and masterful survey of the application of nuclear magnetic resonance spectroscopy and related methods to the assignment of conformation for various peptides in solution. The second by James P. Scannell and David L. Pruess considers a very interesting class of natural products, the amino acid and oligopeptide antimetabolites, which have some chemotherapeutic use. The third by Dennis E. Brown stresses the chemical side of the dioxygenases with special reference to their possible application in synthetic organic chemistry and practical biochemistry.

Much appreciation is due to the authors for their efforts and any failings in the current book must be attributed to the editor. Finally, I wish to thank Professor Sir Ewart Jones and Dr. G.T. Young for the hospitality extended to me at The Dyson Perrins Laboratory, Oxford University, during the Trinity term 1974 when the final editing was done on this book.

Boris Weinstein

Seattle, Washington
July, 1974

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CHAPTER 1
CONFORMATIONS OF PEPTIDES IN SOLUTION AS DETERMINED BY NMR SPECTROSCOPY AND OTHER PHYSICAL METHODS

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I. INTRODUCTION

For many years there has been much interest in the structure of small peptides, but only recently has much work been done on the conformation of these peptides in solution. Though little evidence was available, the general concept regarding the structure and conformation of small peptides was that these compounds existed as a large number of rapidly interconverting conformations in solution at ordinary temperatures. More recent evidence suggests that although this general picture is true for many peptides, some simple peptides often have relatively restricted, and in many cases, fairly well defined conformations in solution. Much of the data that have been accumulated have been the result of studies utilizing various physical methods. These studies are of considerable interest from several points of view. Firstly, of primary interest is the search for a preferred conformation (or a few conformations)

that could be distinguished from the many possible conformations for a particular peptide. Secondly, at the same time it is anticipated that if an understanding of the forces and features that determine the structure and conformation of these low molecular weight and relatively "simple" peptides can be obtained, this could provide a framework for understanding the structure, conformation and function of the more complicated proteins. Thirdly, it is well known that many of these smaller peptides possess a wide variety of biological activities including compounds that function as hormones, antibiotics, toxins, antidotes, ionophores, etc. It is a hope and assumption that there existed a relationship between the conformation of these peptides in solution and their biological activities. If these relationships can be obtained it is possible that this information can provide deeper insight into possible structural modifications which will afford peptides with new or more desirable biological, pharmacological, or medicinal properties. Fourthly, it is hoped that if preferred conformations can be found for these peptides, the peptides will provide models for testing new methods to determine the conformation of peptides by theoretical calculations.

As suggested by the title, the scope of this chapter will be concerned with studies on the conformation of *peptides* in solution, as deduced by NMR and other physical methods. In general, this will encompass linear and cyclic peptides containg from 2 to 12 amino acid residues. While the limitation is rather arbitrary and several exceptions will be included, this range of peptides provides a convenient cutoff and eliminates from the discussion a number of possible topics that have been adequately discussed and reviewed elsewhere. For example, the configuration and rotamer populations of simple amino acids in solution has been discussed [1,2]. Moreover, several articles and books on various aspects of studies of the conformation of polyamino acids have appeared [3-9]. There have also been a number of interesting papers regarding approaches to studying various aspects of protein structure and function in solution (see Refs. [10-13,23]). Excellent surveys of

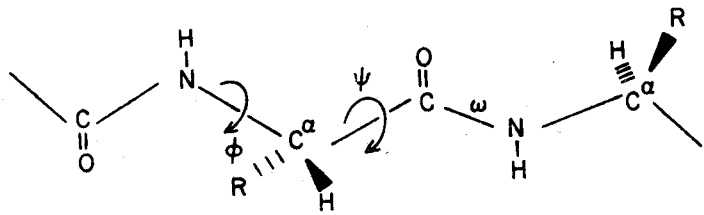
methods for description of peptide conformation and various theoretical approaches for determining conformation also have appeared [14-18]. A number of books, monographs, and reviews specifically devoted to the various theoretical and spectroscopic methods used to study amino acid, polypeptide, and protein conformation are available (see Refs. [1,2,6,7,9,12-29]). In this chapter then, we will try to review critically the results and conclusions regarding peptide conformation that have appeared, and to indicate the problems and unanswered questions that remain.

The principal methods used to study the conformations of peptides in solution have been nuclear magnetic resonance (NMR) spectroscopy, optical rotatory dispersion (ORD), circular dichroism (CD), and infrared (IR) spectroscopy. These studies have often been supplemented and complimented by X-ray diffraction studies or both, and by theoretical calculations. The X-ray studies, of course, are not solution studies. However, they do give much useson of results from solution studies and results from those found in the crystalline state. For study of solution conformation, spectroscopic studies are also often supplemented by various other physical studies. Perhaps the most prominent of these are: (a) hydrogen exchange studies to provide information on hydrogen bonding and shielding of exchangeable hydrogens; (b) thin-film dialysis studies for information regarding the relative sizes and shapes of peptides; (c) dipole moment measurements for geometrical information; and (d) partition chromatography to indicate relative shape and size. Mention will be made of the use of these and other methods as the various studies on peptide conformation are discussed. Energy calculations, often used in conjunction with spectral and other physical evidence, have also been extremely useful in many conformational studies and show great promise for future work. Thus far, energy calculations without some experimental structural information to provide a starting point have not been very successful.

Reviews of some aspects of the study of conformations of short peptides in solution have appeared (see Refs. [1,2,7,8,17,20,22,26-29]).

II. CONFORMATIONS OF SHORT LINEAR AND CYCLIC PEPTIDES

In discussing the conformation of peptides in solution, we are interested in both the overall topochemical picture and the local (individual residue) conformation. In the former category, one might include such information as whether the molecules possess α-helical structure; β-helical structure; intramolecular hydrogen bonds that restrict conformational possibilities; symmetry; hydrophobic, and hydrophilic areas; and other gross conformational features. As we will see, information regarding these features can often be arrived at through model building utilizing information from various spectroscopic investigations such as IR, NMR, ORD, CD, and other spectroscopic methods, and information from various physical studies such as thin film dialysis, gel filtration, hydrogen exchange, and others. Ultimately, however, one would like to obtain information regarding all of the bond angles in the peptide. For this data we refer to the polypeptide backbone conformation and the rotamer populations of the side chains on each amino acid. To specify the secondary structure of a peptide, knowledge of all residue rotational angles ϕ, ψ, ω, χ, is needed [30,31]. A schematic representation of a portion of an all L peptide chain is shown in structure (1) in the planar-zigzag all *trans* form. Unfortunately, two nomenclature systems have arisen for defining these



(1)

torsional angles. In the earlier system suggested by Edsall et al [30], the configuration shown in the above structure was defined as $\phi = \psi = \omega = 0°$ while in the latter nomenclature system of Kendrew et al [31], $\phi = \psi = \omega = 180°$. In general, however, the *trans*

peptide bond is found, and hence this angle is 0° in the former scheme and 180° in the latter nomenclature scheme. A comparison of the ϕ and ψ angles for the two systems is shown in Table 1 at 60° intervals. As can be seen, for peptides containing L amino acids and possessing a *trans* peptide bond, torsional angles from the earlier system can be transformed into the new system by subtracting 180°, and vice versa. For full particulars, one should refer to references [30] and [31]. In our discussion we shall use primarily the earlier nomenclature system of Edsall et al.

A. Linear Dipeptides

General Considerations (Conformational Determinations)

The earliest work on dipeptide conformation came from conformational calculations and X-ray crystallography without particular attention to conformation in solution. There is no reason to assume *a priori* that conformations of small peptides in the crystalline state will be the same as those in solution since crystal forces and solvent-solute interactions may be expected to have differing effects on the conformation of a small flexible molecule. However, X-ray analysis has been most useful in the development of working concepts of peptide structure, especially since it has supported the validity of the near planar peptide bond and has provided bond lengths for the peptide backbone as well as other parameters such as the S-S bond length and the dihedral angle of -C-S-S-C- bridge [32]. These and other data concerning the X-ray of dipeptides and larger peptides has been reviewed by Kendrew and Perutz [33], by Rich and Green [34], and by Marsh and Donahue [35].

The first calculations of allowed conformations reported for a peptide were made for "dipeptides" of the type Gly-Gly and Gly-Ala [36] using the so-called hard-sphere potential model. These kinds of calculations have been greatly refined in recent years and have been extended to include calculations of peptide, polypeptide, and

Table 1

Main Chain Torsion Angles for Various Conformations in Peptides of L-Amino Acids Using Alternate Nomenclature Schemes

ϕ^a	ϕ^b	Rotation about N-C^α		ψ^a	ψ^b	Rotation about C^α-C'	
180°	0°	C^α-C' *trans*	to NH	180°	0°	C^α-N *trans*	to C-O
240°	+ 60°	C^α-H *cis*		240°	+ 60°	C^α-R *cis*	
300°	+120	C^α-R *trans*		300°	+120°	C^α-H *trans*	
0° = 360°	+180°	C^α C' *cis*		0° = 360°	+180°	C^α-N *cis*	
60°	-120°	C^α-H *trans*		60°	-120°	C^α-R *trans*	
120°	- 60°	C^α-R *cis*		120°	- 60°	C^α-H *cis*	

[a] Scheme of Ref. [30].

[b] Scheme of Ref. [31].

protein conformations [6,14-17]. A general approach for doing these conformational energy calculations involves summing the intrinsic threefold torsional potentials about the N-C^{α}(ϕ) and C^{α}-C' (ψ) bonds, the nonbonded steric repulsions, and London dispersion energies (6-12 potential), and the nonbonded monopole-monopole electrostatic interactions for individual residues in the peptide for a particular conformation. Many different conformations of the compound in question are systematically examined to find the lowest energy conformation(s). Studies on similar compounds using molecular orbital calculations have also been used recently [37-43]. Excellent reviews of the methodology for these calculations and their applications to the determination of peptide backbone conformations, corresponding to energy minima for dipeptides and large polypeptides and proteins, have appeared also [6,14-17]. Of course the conformation of the side-chain groups is important, and extensive reviews of the conformations of these groups as they exist in amino acids and peptides have also appeared (Refs. [1,44,45]).

Spectroscopic studies of the conformation of dipeptides in solution were perhaps initiated by NMR studies of glycine oligomers including glycylglycine [46-49]. The use of NMR spectroscopy for determination of solution peptide conformation can be particularly valuable for the following reasons (we will emphasize the uses of proton magnetic resonance (^{1}H NMR) spectroscopy at this juncture):

1. Proton resonance chemical shifts. In principle it should be possible to assign all hydrogen resonances to a particular proton (or protons) in the peptide for subsequent evaluation. For assignment of ^{1}H NMR resonances in peptides, various regions of the spectrum may be assigned to specific kinds of protons of a peptide. For example, the peptide amino proton resonances are generally found in the range from 6.5 to 8.5 ppm downfield from the usual standard tetramethylsilane; most aromatic resonances are also usually found in this region. The α-proton resonances usually occur between about 4 and 5 ppm, and the β as well as the γ, δ, and other proton resonances are usually found between 0.7 and 4 ppm.