ADVANCES IN PROTEIN CHEMISTRY

EDITED BY

M. L. ANSON
Lever Brothers Company Research Center
Edgewater, New Jersey

KENNETH BAILEY
University of Cambridge
Cambridge, England

JOHN T. EDSALL
Harvard Medical School
Boston, Massachusetts

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CONTRIBUTORS TO VOLUME VIII

JETT C. ARTHUR, JR., *Southern Regional Research Laboratory, New Orleans, Louisiana*

HENRY BORSOOK, *Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California*

E. BRICAS, *Laboratoire de Chimie Biologique de la Faculté des Sciences, Paris, France*

PER FLODIN, *Institute of Biochemistry, University of Uppsala, Sweden*

CL. FROMAGEOT, *Laboratoire de Chimie Biologique de la Faculté des Sciences, Paris, France*

ERNEST F. GALE, *Medical Research Council Unit for Chemical Microbiology, Department of Biochemistry, University of Cambridge, England*

FRANK W. PUTNAM, *Department of Biochemistry, University of Chicago, Chicago, Illinois*

ARNE TISELIUS, *Institute of Biochemistry, University of Uppsala, Sweden*

GREGORIO WEBER, *Sir William Dunn Institute of Biochemistry, University of Cambridge, England*

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Naturally Occurring Peptides

By E. BRICAS and Cl. FROMAGEOT

Laboratoire de Chimie Biologique de la Faculté des Sciences, Paris

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INTRODUCTION

Peptides are generally classified as being between free amino acids at the one extreme and proteins at the other, and they possess a spectrum of properties intermediate between the properties of amino acids and of proteins. The systematic study of peptides is not very well developed, and a reason for this is the considerable difficulty often found in isolating them from their natural media, in separating them from one another, and in obtaining them in a pure state. Whereas numerous articles have been concerned with amino acids and proteins, and certain peptides (glutathione, penicillins, and peptides of rye ergot, etc.) have been the subject of various reviews, little has as yet been published on peptides as a whole. A single study by Synge (575) deals exclusively with naturally occurring peptides and correctly emphasizes their interest.

1. *Definition of Naturally Occurring Peptides*

Peptides, resulting as they do from the combination of a relatively small number of amino acids, are obviously distinguished from them by their molecular weights and by the fact that these peptides can be resolved into their constituent amino acids by hydrolysis. For the most part, their chemical reactions, physicochemical properties, e.g., ultraviolet spectrum (51) are similar to those of the amino acids from which the peptides are built. However, as the number of constituent amino acids increases, the peptides take on properties which approach those of true proteins. In order to distinguish peptides from proteins, it is frequently convenient to use the size of the molecule, i.e., its molecular weight, as a criterion. Arbitrarily, as Synge (575) has proposed, we can consider as peptides such substances whose molecular weight is below 10,000, the maximum molecular weight of substances still capable of diffusing across an ordinary cellophane membrane. This characteristic, arbitrary though it be, is generally accepted as the criterion.

Peptides thus defined also differ from proteins in that peptides do not seem to undergo denaturation. Furthermore, peptides are generally composed of a much smaller number of different kinds of amino acids than are found in proteins. However, these criteria for classification are not absolute, for, on the one hand, it is generally accepted that the polymer of D-glutamic acid, extracted from capsules of *Bacillus anthracis* (p. 64), be considered a peptide, in spite of its high molecular weight (of the order of 50,000), because it is composed of only one type of amino acid. On the other hand, all workers agree to classify as proteins certain substances which are composed of only a few different kinds of amino acids, e.g., silk fibroin, 83% of which is composed of only four kinds of amino acids, tyrosine, serine, glycine, and alanine, whereas certain characteristic peptides contain up to ten and even more diverse amino acids.

In addition to or in place of the classical peptide bond (between the α-carboxyl group of one amino acid and the α-amino group of another), certain naturally occurring peptides have what we shall call a "peptidoid" bond. Such peptidoid bonds exist between the groupings of two amino acids of which neither or only one is in the α-position, for example in the peptides carnosine, glutathione, and pteroyl glutamic acid. Moreover, since peptides possess still other types of linkages or bonds, one may define a peptide as any substance built up of, or at least containing, two amino acids linked either by a peptide or peptidoid bond, and satisfying the conditions already described above. Different peptides may then be classified according to the number of amino acids linked by peptide or peptidoid bonds. This definition excludes compounds composed of a single amino acid combined with one or more other substances that are not amino acids, such as hippuric, pantothenic, and glycocholic acids, biocytin (biotinyl-ϵ-lysine), *Lactobacillus bulgaricus* factor (pantethine), coenzyme A and other substituted amino acids, with which we shall not deal.

A priori, peptides can be classified as arising from different origins. One kind of peptide results from the partial degradation of proteins under the influence of various hydrolytic agents, such as enzymes, acids, and bases. The characterization of such peptides is fundamental for the understanding of the structure of proteins. In a second category are naturally occurring peptides found either in cells or tissues, or in the secretory or excretory products of different species of animals and plants. In this classification, certain peptides could be considered only as fragments of proteins formed in the process of hydrolysis or synthesis[1] and sometimes

[1] Probably certain as yet undefined peptides belong to this group; their presence has been noted in blood (120,121), in various organs of higher animals (85,119,120,124, 125), in urine (122,123,164,186,506), in higher plants (239,242,440,441,599), and in culture media in which bacteria have been grown (479).

TABLE I

Uncommon Amino Acids Found in Naturally Occurring Peptides

Principal carbon chain	Amino acid	Peptide	References
C_2	Sarcosine	Actinomycin C	(93,94,156)
C_3	D-Alanine	Peptide from penicillin treated *S. aureus* cells	(446,450)
	β-Alanine	Carnosine	(237)
		Anserine	(13)
	α-hydroxyalanine	Ergotamine	(564,568)
		Ergosine	
	D-Serine	Polymyxin D	(55,541)
	α,β-Diaminopropionic acid	Viomycin	(261)
	D-β,β′-Dimethylcysteine	Penicillins	(409)
	Mesolanthionine	Subtilin	(17)
C_4	D-Glutamic acid	Polypeptides from the capsules of *B. anthracis*, and of the bacteria of *B. mesentericus-subtilis* group	(313,314)
		Peptide from penicillin treated *S. aureus* cells	(450)
	L-α,γ-Diaminobutyric acid	Polymyxin A (Aerosporin)	(55,114,541)
		Polymyxins B, C, D, E	
		Circulin	(458)
		Polypeptin	(290)
	L-Allohydroxyproline	Phalloidin	(626)
		α- and β-Amanitins	(627)
	Pyrrolidonecarboxylic acid[a]	Tripeptide from *Pelvetia fastigiata*	(163)
		Eisenin	(440,441,442)
C_5	D-Valine	Gramicidin A	(574)
		Actinomycin C	(93,94,156)
	α-Hydroxyvaline	Ergocristine	(564,568)
		Ergocryptine	
		Ergocornine	
	N-Methyl-L-valine	Actinomycin C	(93,94,156)
	L-Ornithine	Tyrocidine	(227)
		Gramicidin S	(573)
C_6	D-Leucine	Gramicidin A	(388)
		Polymyxin A (Aerosporin)	(114,327)
		Polymyxin B	(327)
		Polymyxin D	(55,327)
		Polymyxin E	(326)
		Circulin	(458)
	D-Isoleucine	Bacitracin	(143)
	D-Alloisoleucine	Actinomycin C	(94)
	β,ε-Diaminocaproic acid (isolysine)	Streptothricin Viomycin	(111,112) (111,112,261)

TABLE I (*Continued*)

Principal carbon chain	Amino acid	Peptide	References
	β,ε-Diaminocaproic acid	Streptolin	(112,530,598)
	L-Methylhistidine	Anserine	(385)
C_7	α,ε-Diaminopimelic acid	Peptide attached to a lipopolysaccharide isolated from *M. tuberculosis*	(30)
	$C_5H_8S(NH_2)_2(COOH)_2$	Subtilin	(109)[b]
C_6-C_3	D-Phenylalanine	Tyrocidine	(227)
		Gramicidin S	(573)
C_8-C_3	Tryptophan derivative	Phalloidin	(626)
		α- and β-Amanitins	(627)
	p-Aminobenzoic acid	Peptides of folic and folinic acids; peptide of *p*-aminobenzoylpolyglutamic acid	(22,73,303, 304,425,461, 462,491,492, 555,556)

[a] It is possible that this acid is not found in the naturally occurring peptide and is only an artefact obtained during the isolation of the peptide.

[b] See also G. Alderton. (1953). *J. Am. Chem. Soc.* **75**, 2391.

accordingly having only a more or less ephemeral existence. On the other hand, within this same classification, there are more or less stable peptides synthesized directly from amino acids and, when necessary, from other simple constituent molecules. Finally, we are faced with the question as to whether naturally occurring peptides in the free state are present in the living cell in the same form as isolated in the laboratory, or whether they are constituent parts of protein molecules. If they are of protein origin, they might represent an easily detachable prosthetic group, or perhaps be the more resistant fragments of a particularly labile molecule, whose peptide fragments appear to be in a free state owing to the techniques employed in their isolation. This question has arisen particularly for hypertensin, the ACTH peptides, ocytocin, and vasopressin. We designate as naturally occurring peptides those believed to exist as such in the cell, or at least easily isolatable as such. Moreover, it is not always possible to establish a clear line of distinction between such peptides and those which, as yet poorly characterized, seem to be only intermediary products in the synthesis or degradation of proteins. In the present review, we shall discuss only naturally occurring peptides, as defined above.

Naturally occurring peptides are of great interest, since amino acids form the building blocks not only for the synthesis of proteins but also for a whole series of peptides which often possess extremely potent biological activity such as antibiotics, hormones, toxins, and growth factors.

2. *Homeomeric and Heteromeric Peptides*

The distinction between naturally occurring peptides existing as such and peptides which are only fragments of protein undergoing degradation or synthesis is based not only upon strikingly characteristic linkages between amino acids, but also upon the frequent occurrence of uncommon amino acids and other substances which have not been found in proteins (Table I). We propose to designate as *homeomeric*[2] peptides those which are made up only of amino acids, whatever their nature, and as heteromeric peptides those which contain, besides amino acids, one or more other molecules (Table II).

TABLE II

Molecules Other than Amino Acids Found in Naturally Occurring Heteromeric Peptides

Other molecules	Peptide	References
Ethanolamine	Gramicidin	(572)
Lactic acid	Lycomarasmin	(654)
Δ-β,γ-*n*-Hexenoic acid	Penicillins	(116)
Δ-γ,δ-*n*-Hexenoic acid		
n-Octanoic acid		
Phenyl acetic acid		
Parahydroxyphenylacetic acid		
(+)6-Methyloctanoic acid	Polymyxins; probably also circulin and polypeptin	(630)
Lysergic acid	Peptides of rye ergot	(316,317)
2-Amino-4-hydroxy-6-methyl-pteridine	Folic acid and derivatives	(22,303,304, 425,462,555, 556)
2-Amino-4-hydroxy-5-N-formyl-5,6,7,8-tetrahydro-6-methylpteridine	Folinic acid and derivatives	(274,348,505)
Uridine-5'-pyrophosphate attached to an N-acetyl amino sugar	Peptide from penicillin treated *S. aureus*	(448,450)
Hydronaphthalene nucleus combined with glucuronic acid + phosphoric acid	Viscotoxin Actinomycin A	(634) (612)
Quinoid chromophore	Actinomycin C	(94)

3. *Structure of Peptides*[3]

Naturally occurring peptides show various types of structure. *Linear* peptides obviously possess two ends, one of which is made up of an amino

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[2] These terms, homeomeric peptides and heteromeric peptides, should not be confused with the terms, *homopeptide* and *heteropeptide*, which Blout and Linsley (77) used to designate peptides consisting respectively of a single type of amino acid or of several different types of amino acids.

[3] The different techniques used for the study of peptide structure have been reviewed in a preceding volume (502).

acid whose —NH_2 group is free, or at least is not linked in a peptide bond. We call this the *initial* amino acid. The other end consists of an amino acid whose —COOH group is free, or at least is not linked in a peptide bond. We call this the *terminal*[4] amino acid. In the case of the linear peptides, the number of peptide bonds is one less than the number of amino acids.

In addition to linear peptides, one can envisage the existence of *cyclic* peptides in which there is neither an initial nor terminal amino acid and for which the number of peptide bonds equals the number of amino acids. Of course, one can also postulate the existence of partially cyclic peptides, forming sigma-shaped chains (σ), in which either the terminal or initial amino acid is internally linked in the peptide. Therefore, it is possible to have partially cyclic peptides possessing either a terminal (6^{COOH}) or an initial amino acid (${}_{\mathrm{H_2N}}9$). Certain peptides possess other structures, e.g., bicyclic and polycyclic, owing to the presence of nonpeptide linkages between amino acids, in addition to the characteristic α-peptide bond. Examples of such structures are penicillins, peptides of rye ergot.

4. *Biological Origin of Naturally Occurring Peptides*

As far as their biological origin is concerned, peptides can be grouped in two categories. Those in the first category, like glutathione, are widely distributed in nearly all living cells, regardless of kind, e.g., organs of higher animals, higher plants, yeast, and bacteria. The second category, in contrast, is composed of peptides which are found only in certain organs of higher animals, or are synthesized only by certain well-defined species of organisms. Thus, ocytocin and vasopressin are found mainly in the posterior lobe of the hypophysis of higher animals, viscotoxin is peculiar to mistletoe (*Viscum album*), phalloidin and its accompanying toxins are found only in amanites (*Amanita Phalloides*), and the various groups of antibiotic peptides are synthesized only by well-defined groups of microorganisms. In view of the fact that our present knowledge concerning the structure of naturally occurring peptides is still insufficient to provide a basis for their rational classification, we shall, in the present review, employ a classification based upon their biological origin.

5. *Uncommon Amino Acids Found in Naturally Occurring Peptides*

Some naturally occurring peptides, as we have said previously, contain certain uncommon amino acids. It should be noted here that the

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[4] The expressions, initial and terminal amino acid, seem to us to be preferable to amino acid with a free terminal NH_2, or free terminal COOH. Actually, naturally occurring peptides are known in which the initial amino acid does not possess the free NH_2 group, as this amino acid has substituted on its nitrogen certain radicals, e.g., the peptides of rye ergot (p. 78) in which the nitrogen of the initial amino acid is linked to lysergic acid. An analogous comment may be made concerning the COO H group of the terminal amino acid.

adjective "uncommon" can have only a relative and temporary meaning, since it is justified only inasmuch as these uncommon amino acids have not as yet been found to be constituents of ordinary proteins. Nevertheless, it seems certain that such radicals, since they form the foundation of naturally occurring peptides, are in a large measure responsible for the characteristic biological properties of these peptides. The uncommon amino acids of naturally occurring peptides can be classified in two categories: (1) those which are simply optical antipodes of the usual amino acids and are distinct only in that they belong to the D series; (2) those which have not as yet been found to be constituents of ordinary proteins and may belong to either the D or L series. Table I gives a list of these uncommon amino acids that are found in various naturally occurring peptides.

The dual presence, of linkages other than the classical α-peptide bond, and of uncommon amino acids in numerous naturally occurring peptides, enables us to predict that, if enzymes exist which hydrolyze such peptides, these enzymes should differ by their specificity from the peptidases which hydrolyze peptides resulting from the partial degradation of proteins. Actually, the supposition is true, for several such enzymes are known, which we shall at least mention in this review.

I. PEPTIDES WITH WIDESPREAD DISTRIBUTION IN LIVING CELLS

1. *Glutathione* (*γ-L-Glutamyl-L-cysteinylglycine*)

Among the naturally occurring peptides which are now known, glutathione is the most widespread, with many studies (37,65,191,586) having been devoted to it. We will briefly recall the main stages in the study of glutathione, and the results of these studies that are today classic. We shall also describe in detail the more recent findings concerning the role and biological behavior of this tripeptide.

a. Structure. After its isolation in 1921 by Hopkins (277), from yeast and from mammalian liver and muscles, the composition of glutathione was established independently by Hopkins (278) and by Kendall, Mason, and McKenzie (345). Its structure as a γ-peptide was hinted at as early as 1923 by Quastel, Stewart, and Tunnicliffe (481). Kendall, McKenzie, and Mason (344,345), returning to the study of the structure of the isolated pure peptide, confirmed that the α-amino carboxyl groups of glutamic acid were free, and they also showed that glycine, with its carboxyl group free, was at the end of the chain. This latter fact was discovered independently by Nicolet (437). These results, based upon chemical methods, were confirmed by studies on the enzymatic degradation of glutathione by peptidases (229). The structure of glutathione as