Synthetic Aspects of Biologically Active Cyclic Peptides

—— Gramicidin S and Tyrocidines

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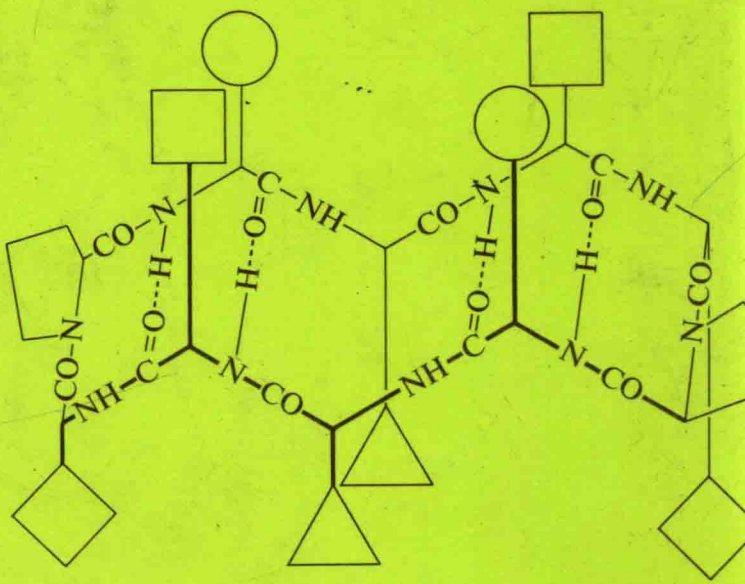

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Preface

Recent years have seen dramatic development in the field of peptide chemistry. The importance of conformations of naturally occurring peptides has been emphasized in order to interpret chemical properties and biological activities, just as in protein chemistry. Ever since V. du Vigneaud and his associates verified in 1953 that the chemical synthesis of complex physiologically active peptides is feasible, a good deal of effort has been devoted by organic chemists to the synthesis of naturally occurring peptides and their structural analogs. Hundreds of analogs of biologically active peptides have been synthesized and studied to determine which structural factors might have the biological effect caused by natural molecule.

Most peptide antibiotics from microbial origin have characteristic features and various biological activities. Many of the known peptides are cyclic and contain unusual amino acids, D-amino acids or hydroxy acids in their molecules. Although recent progress in instrumental analysis has led us to make sure of their primary structures and/or conformations in high sensitivity and accuracy, it is still valuable to study synthetic analogs in order to gain much precise information on the structure-function relationships in natural peptides, because structures of the natural peptide antibiotics are often very complexed.

Comprehensive reviews and systematic treatises stressing the organic chemistry of biologically active cyclic peptides such as gramicidin S are still few. In writing this book we attempt to classify a number of microbial peptides into several groups according to their activities and to the type of structure in Chapter 1. Chapter 2 provides a historical summary on isolation and primary structure of gramicidin S and the related peptides such as, tyrocidines, linear gramicidins, gramicidin J and gratisin.

Chapters 3 and 4 focuss on detailed descriptions on chemical aspects of gramicidin S, tyrocidines and their analogs from the viewpoint of their organic synthesis and structure-function relationship. Chapter 5 covers various recent studies on the conformation of the peptide antibiotics by many methods including X-ray, ORD, CD, NMR and IR, and discusses some problems concerning the conformation and the topochemistry of gramicidin S and its analogs. Chapters 6 and 7 deal with mechanism of their antibacterial action and biosynthesis of gramicidin S and tyrocidines. Several topics on organic and biological chemistry of phytotoxic AM-toxins are presented in Chapter 8.

Finally, we should like to acknowledge our co-workers who have discussed, criticized and so helped the development of our ideas over the years. We are also indebted to Miss A. Okabe for typing and Mr. M. Takahatake of Kodansha Scientific who prepared the work for the printer.

Fukuoka
Saga

June, 1979

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Abbreviations

The abbreviations suggested for amino acids and peptides (*J. Biol. Chem.*, **247**, 977 (1972)), rules for naming synthetic modifications of natural peptides (*ibid.*, **242**, 555 (1967)) and symbols for the description of the conformation of polypeptide chains (*ibid.*, **245**, 6489 (1970)) are those recommended by the IUPAC–IUB Commission on Biochemical Nomenclature. Unless otherwise specified, the amino acids and peptides are the L stereoisomers. Additional and frequently used abbreviations are as follows:

Ac	Acetyl
AcOEt	Ethyl acetate
Ahp	2-Amino-5-(*p*-hydroxyphenyl)pentanoic acid
Aib	α-Aminoisobutyric acid
βAla	β-Alanine
Amp	2-Amino-5-(*p*-methoxyphenyl)pentanoic acid
Aoe	2-Amino-8-oxo-9,10-epoxydecanoic acid
App	2-Amino-5-phenylpentanoic acid
ATP	Adenosine 5′-triphosphate
δ-Ava	δ-Aminovaleric acid
BBI	Bowman-Birk inhibitor
Boc	*t*-Butyloxycarbonyl
CD	Circular dichroism
C-G	Cytidine-guanosine
Cha	Cyclohexylalanine
Dbu	2,4-Diaminobutyric acid
DCC	Dicyclohexylcarbodiimide
Dha	Dehydroalanine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DNP	2,4-Dinitrophenyl
EA	Ethanolamide
EDC	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (water-soluble carbodiimide)
EPR	Electron paramagnetic resonance
GS	Gramicidin S
H_2/Pd	Catalytic hydrogenation
HE	Heavy enzyme

Hmb	2-Hydroxy-3-methylbutanoic acid
Hmp	2-Hydroxy-4-methylpentanoic acid
HOBt	1-Hydroxybenzotriazole
HONSu	*N*-Hydroxysuccinimide
Hyp	Hydroxyproline
IR	Infrared
IE	Intermediate enzyme
LE	Light enzyme
MA	Mixed anhydride
MBzl, MeOBzl	*p*-Methoxybenzyl
mp	Melting point
Ms	Methanesulfonyl
Mz	*p*-Methoxyphenylazobenzyloxycarbonyl
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
Nva(4OH)	4-Hydroxynorvaline
OBzl	Benzyl ester
OEt	Ethyl ester
OMe	Methyl ester
ONp	*p*-Nitrophenyl ester
ONSu	*N*-Hydroxysuccinimide ester
ORD	Optical rotatory dispersion
Pht	Phthaloyl
Pip	Pipecolic acid
PMR	Proton magnetic resonance
PPi	Pyrophosphate
Pro(4F)	4-Fluoroproline
RNA	Ribonucleic acid
SPS	Solid-phase synthesis
Suc	Succinyl
TA	Tyrocidine A
TCA	Trichloroacetic acid
TFA	Trifluoroacetic acid
TLC	Thin-layer chromatography
Tos	Tosyl, *p*-toluenesulfonyl
Trt	Trityl, triphenylmethyl
Tyr(Me)	*O*-Methyltyrosine
UV	Ultraviolet
Z	Benzyloxycarbonyl
$Z(NO_2)$	*p*-Nitrobenzyloxycarbonyl
Z(OMe)	*p*-Methoxybenzyloxycarbonyl

Contents

CHAPTER 1

Introduction

Biologically active peptides cover a wide range from dipeptide derivatives, such as penicillin, to polypeptides consisting of over fifty amino acid residues, such as cobrotoxin. Recently, peptides possessing unique activities have been found. For instance, several hormone-releasing hormones and prohormones were obtained from animals, and their structures and functions have been studied. On the other hand, antibiotic or toxic peptides produced by microorganisms are also of interest. Unlike peptides from animals, these microbial peptides contain not only the usual L-amino acids found in proteins but also often D-amino acids, unusual amino acids, hydroxy acids and many other components.

Table 1.1 lists a number of microbial peptides classified into several groups according to their activities. As most studies on natural peptides have been devoted to searches for new substances possessing antimicrobial activity, it is not surprising that many antibacterial, antiyeast or antifungal peptides are included in Table 1.1. Recently, peptides showing unique activities towards insects or plants have been obtained and these are also listed in Table 1.1.

Many other interesting substances have also been found. For example, beauvericin is toxic to the brine shrimp, chlamydocin is cytostatic to mastocytoma cells, and surfactin is a surfactant and has clotting inhibitory activity in the thrombin-fibrinogen reaction. Several proteinase-inhibitory peptides of microbial origin presented in Table 1.1 are very different from those of plant or animal origin in that the microbial peptides generally have a low molecular weight and contain unusual constituents.

On the other hand, the peptides derived from microorganisms can also be grouped by the types of structure, as summarized in Table 1.2. Firstly, they can be divided roughly into linear and cyclic molecules. Secondly, the cyclic peptides can be divided into five types as follows. (1) Low molecular cyclic

TABLE 1.1 Activities and Names of Microbial Peptides

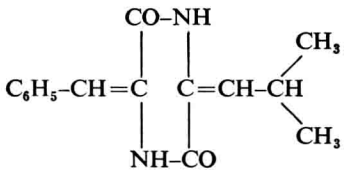
Activity	Name
1) Antibacterial	actinomycins, alamethicin, bacitracin, capreomycins, echinomycin, enniatins, etamycin, gramicidin A, gramicidin S, monamycin, nisin, polymyxins, serratamolide, subtilin, triostins, tyrocidines, valinomycin
2) Antitumor	alazopeptin, albonoursin, duazomycin B, neocarzinostatin
3) Antiyeast, antifungal	amidomycin, mycobacillin, neoantimycin, stendomycin
4) Insecticidal	aspochracin, destruxins
5) Phytotoxic, plant growth inhibitory, chlorosis-inducing	AM-toxins, tabtoxins, Cyl-2, malformin tentoxin
6) Proteinase inhibitory	antipain, bestatin, chymostatin elastinal, leupeptins, pepstatins
7) Iron-transport, siderochrome	enterobactin, ferrichrome

TABLE 1.2 Types and Names of Microbial Peptides

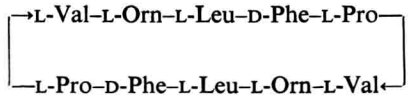
Type	Name
1) Linear	cerexin, gramicidin A, leupeptin, neocarzinostatin, pepstatin
2) Cyclic	
a) Low molecular cyclic peptide	albonoursin, D-cycloserine, penicillin
b) Simple cyclic oligopeptide	antamanide, Cyl-2, fungisporin gramicidin S, malformin, tentoxin, tyrocidines
c) Cyclic oligopeptide having peptidyl side chain	bacitracin, bottromycin, capreomycin, colistin, polymyxins
d) Cyclic peptolide	amidomycin, AM-toxins, destruxins, enniatins, monamycin, serratamolide, sporidesmolide, valinomycin
e) Cyclic peptidelactone	actinomycins, etamycin, stendomycin, vernamycin, viscosin

peptides consisting of amino acid or dipeptide derivatives; the structure of albonoursin (**1.1**) is shown as an example. (2) Simple cyclic oligopeptides containing several amino acids which are linked through a peptide bond;

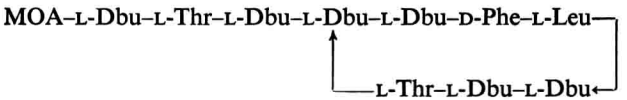
gramicidin S (**1.2**) is an example. (3) Cyclic oligopeptides having a peptidyl side chain, which can be formed if a peptide of type (2) contains an acidic or basic amino acid residue(s); polymyxin B_1 (**1.3**) is an example. (4) Cyclic peptolides which have one or more hydroxy acid residues in addition to amino acid residues as constituents and therefore have an ester bond in the ring structure; AM-toxin I (**1.4**) and destruxin B (**1.5**) are examples. (5) Cyclic peptidelactones, in which a hydroxyamino acid(s) is present and the hydroxy group instead of the amino group participates in the formation of the backbone structure; viscosin (**1.6**) is an example.



(**1.1**)

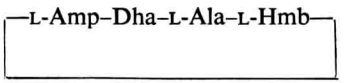


(**1.2**)



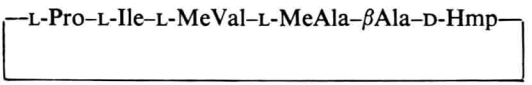
MOA, (+)-6-methyloctanoic acid

(**1.3**)



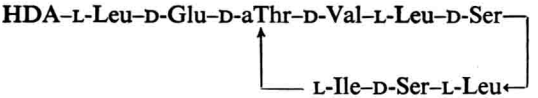
(**1.4**)

Amp, 2-amino-5-(*p*-methoxyphenyl) pentanoic acid;
Dha, dehydroalanine;
Hmb, 2-hydroxy-3-methylbutanoic acid



Hmp, 2-hydroxy-4-methylpentanoic acid

(**1.5**)



HDA, (−)-3-hydroxydecanoic acid

(**1.6**)

Among the wide variety of peptide antibiotics, gramicidin S has been one of the most extensively studied by various physico- and organochemical procedures. The reason for this is that its primary structure was determined early in the 1940s, it is available as a pure species from the natural source in fairly amounts, and it has a unique and attractively complex cyclic structure.

In this book, we will describe the chemistry of gramicidin S and related compounds such as tyrocidines, as well as AM-toxins. We have made substantial contributions to the synthetic chemistry of these cyclic peptides, and this book will deal especially with the chemistry of those natural cyclic peptides and analogs synthesized in our laboratory, from the viewpoint of their chemical synthesis and structure-function relationships. There have been reviews on the synthetic and biological aspects of gramicidin S.[1,2]