

One Hundred Years of Peptide Chemistry*

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Emil Fischer is considered to be the founding father of the field of peptide chemistry and originator of the term peptide. In the beginning of the 20th century, Fischer had a clear vision to foresee the day when a protein would be synthesized. Subsequent to this brilliant beginning, progress however was slow for the next 50 years. In 1953, the chemical synthesis of oxytocin by duVigneaud was a landmark achievement. The subsequent decades witnessed the discovery of a vast number of biologically active peptides. The last three decades saw development of multiple simultaneous syntheses, peptide libraries and peptide vaccines. This article is a concise account of the types of peptides discovered and their biological role.

Introduction

In 1901, Emil Fischer (with E Fourneau) published an article which reported the preparation of the first dipeptide, glycylglycine, obtained by partial hydrolysis of the diketopiperazine of glycine in the laboratory. At the 14th meeting of the German scientists and physicians on September 22, 1902 at Karlsbad, Fischer introduced the name peptides (from *pepsis* = digestion or *pepton* = digestion products of proteins). The term 'peptides' denotes relatively small compounds which resemble proteins except that the latter are higher molecular weight substances. Thus, the molecules built up of 50 or more amino acids are generally regarded as proteins and those containing fewer amino acids are denoted as peptides. The chemical synthesis of peptides, as envisaged by Fischer, involves the reproduction or perhaps recreation of Nature's molecules.

Five years later, in January 1906, in his famous lecture at the Deutsche Chemische Gesellschaft at Berlin, Fischer gave an illuminative review of the efforts of his school in peptide synthesis, which stimulated speculations on the imminent availability of

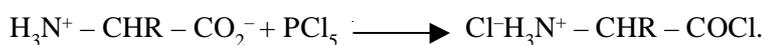
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Fischer, amino acids, proteins, peptides, peptide bond, peptide synthesis.



synthetic living matter. He eventually succeeded (with Axhausen) in synthesizing an impressive octadecapeptide, L-Leu-(Gly)₃-L-Leu-(Gly)₃-L-Leu-(Gly)₈-Gly. Due to the nonavailability of a method for removing the benzoyl or acetyl groups, Fischer employed the first urethane type protecting ethoxycarbonyl group (C₂H₅OCO) with the hope of removing it from the amino nitrogen by reaction with mild alkali. He was able to perform peptide coupling at the carboxyl group, by preparing amino acid chloride hydrochlorides.



Introduction of the amino protecting benzyloxycarbonyl group in 1932 by Max Bergmann and Leonidas Zervas coupled with improvements in the methods of peptide bond formation, culminated in the chemical synthesis of the oligopeptide hormone oxytocin by du Vigneaud in 1953. The discovery of a method for sequencing of amino acids in polypeptides and proteins was an epoch-making event in the history of peptide chemistry. From thereon, the synthesis of peptides progressed by leaps and bounds.

The demand for peptides and proteins is enormous, and is increasing rapidly with time. During the past 35 years the discovery of a vast array of naturally occurring peptides with potent and specific biological activities has further increased their importance. Pharmacological studies required the synthesis of not only the often hard-to-isolate natural peptides, but also of numerous analogs, which led to investigations on the chemical structure – biological activity relationship.

Amino Acids and the Peptide Bond

The monomers present as repeat units in peptides and proteins are α -amino acids (a carboxyl group and an amino group bonded to the same α -carbon). The α -carbon is asymmetric, bonded to four different substituent groups, and is thus a chiral center. Today, 20 genetically encoded amino acids are known as building blocks of peptides and proteins. Almost all of them present in peptides have L-configuration. D-Amino acids have been found only in

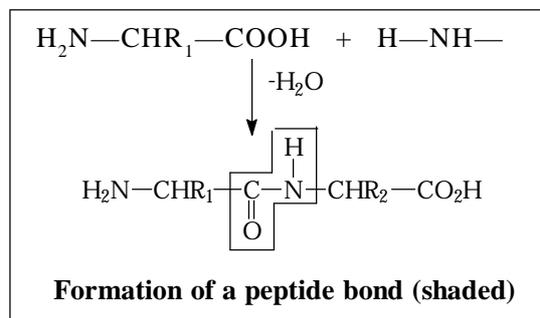
Box 1.

Many large peptides made by Fischer may not meet today's criteria of purity! However, half a century later, 31 out of 34 peptides from Fischer's laboratory (made available by his son H O L Fischer) were found to be pure enough to give a single spot by paper chromatography.

Box 2.

Proteins (from the Greek *protos* = first or *proteios* = primary) are the most abundant biopolymers found in living cells. They are present not only in all cells but also in all components of cells. They occur in many varieties; thousands can be found in a single cell. They exhibit great diversity in biological function.





small peptides of bacterial cell walls, peptide antibiotics and peptides in South American frog skin.

The condensation of two amino acid molecules through covalent linkage results in a dipeptide (amide) bond. Such a linkage is formed by elimination of water from the α -carboxyl group of one amino acid and the α -amino group of another.

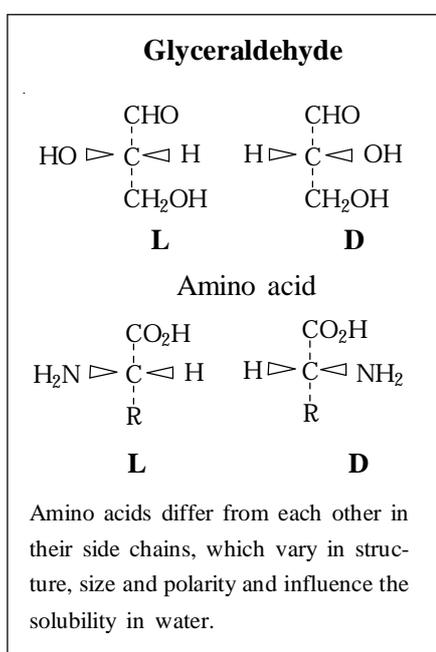
The polypeptide chain can be synthesized by successive condensation of amino acids.

¹ Hormones (derived from the Greek verb *horman*, meaning 'to stir up or excite') are chemical messengers secreted in one part of the body and carried rapidly in the blood to other parts of the body, where they exert influence.

Peptides and Biological Activity

Biologically active peptides range in size from small molecules containing only two or three amino acids to large molecules containing several tens of amino acids. Among them, neuropeptides, hypothalamic hormones¹ (releasing and release-inhibiting hormones), proteohormones of the pituitary, thyroid hormones, gastrointestinal peptides, muramyl peptides, peptides of immunological significance, peptide vaccines, plasma kinins, atrial natriuretic peptides, peptide antibiotics, peptide toxins, peptide insecticides and herbicides are some of the important classes.

Peptide Hormones: Many peptides which are already in clinical use and a few others are described in this section. One of the brain peptides, the thyrotropin releasing hormone (TRH) was the first to be isolated from 1,00,000 pig hypothalami. This is the molecule by



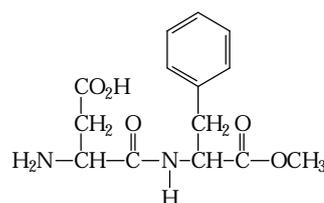
Box 3.

Fred Sanger of Cambridge University used 2,4-dinitrofluorobenzene as a marker for *N*-terminal amino acid of a peptide to elucidate the entire structure of insulin in 1955 employing partial peptide fragment sequences in the manner of jigsaw puzzle for which he was awarded the Nobel Prize in Chemistry in 1958. Unaffected by acclaim, he continued the work with uninterrupted zeal and made an in-depth study of nucleic acids and developed a method for DNA sequencing by the enzymatic method. Recognizing this contribution he became the recipient of the Nobel Prize in Chemistry again in 1980. Thus, Sanger is the only person to receive the Nobel Prize on two occasions in Chemistry.

which the hypothalamus, through the pituitary, regulates the functions of the thyroid gland. Later, the first isolation of 800 mg of luteinizing hormone releasing hormone (LHRH or gonadoliberin, pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) from ventral hypothalami of 1,65,000 pigs was achieved by 12 successive purification steps by Andrew V Schally and Roger Guillemin, who were awarded the Nobel Prize for Medicine and Physiology in 1977. LHRH acts on the pituitary to promote rapid release of LH and follicle-stimulating hormone (FSH), which in turn regulates ovulation and spermatogenesis.

Among several of its clinical applications, LHRH and its analogs find use as a nonsteroidal male and female contraceptive or as fertility agents. Somatostatin, the tetra-decapeptide hormone released by the hypothalamus, plays an important physiological role as an inhibitor for the release of several hormones (glucagon, growth hormone, insulin, gastrin). The octapeptide angiotension II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) causes increase in blood pressure. α -melanocyte stimulating hormone (α -MSH) also called α -Melanotropin (Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂), is secreted in the anterior lobe of the pituitary gland. It exerts its activity at peripheral receptors, which are primarily responsible for its pigmentation properties. The other hormone secreted in the anterior lobe is a 39 residue peptide

Small peptides can have large biological effects.



Artificial sweetener, commercially synthesized, known as Aspartame® or Nutrasweet®. It is about 200 times sweeter than sucrose and possesses a good flavour without unpleasant after-taste. Its importance for weight watchers and diabetics is obvious.

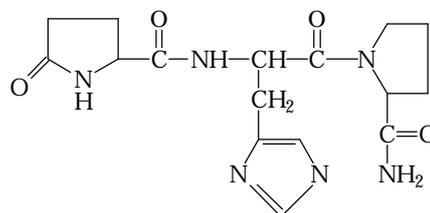
Box 4.

Oxytocin, the first hormone to be isolated, structure determined and synthesized in the laboratory by Vincent du Vigneaud (Nobel Prize in Chemistry in 1955), is clinically used for the stimulation of uterine contraction in cases of labor. It also plays a pivotal role in milk-ejection in lactating glands.

² There is no doubt regarding the exceptional significance of insulin in medicine. It controls the unfortunately rather common deficiency disease diabetes mellitus.

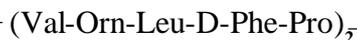
corticotrophin or adrenocorticotropin hormone (ACTH). This finds use in medicine for the treatment of hypophyseal insufficiencies and inflammatory processes. The gastrin family of peptides plays a significant role in gastrointestinal functions including secretion, motility and absorption. Cholecystokinin is an important hormone, which belongs to this family mediating digestive functions and feeding behavior. In the course of the isolation of oxytocin, the second hormone of the pituitary, vasopressin, a peptide with pressor and antidiuretic effects, was also isolated. The deficiency of vasopressin causes diabetes insipidus². Persons affected by it have excessive excretion of water, which is controlled by applying a solution of the peptide in the form of nasal spray. The vital regulation of the level of Ca²⁺ ions in the blood is controlled by parathyroid hormones. These hormones raise the level of calcium concentration in blood by mobilizing calcium uptake from the bones. On the other hand, the thyroid hormone, calcitonin directs the flow of calcium ions in the opposite direction. Thus a constant level of calcium ions is maintained in the blood.

Neuropeptides: There are about 50 neuropeptides whose molecular structures are known; many of them exist within closely related families of molecules. Several endogenous opioid peptides [endorphins, enkephalins, dynorphins, dermorphins, deltorphins, dermorphin gene associated peptide (DGAD)] possessing morphinomimetic properties are very well studied. Enkephalins (meaning isolated from the brain), two pentapeptides (Tyr-Gly-Gly-Phe-Met/Leu) are found in varying amounts in almost all



Primary structure of TRH (pGlu-His-Pro-NH₂)



**Gramicidin S**

Active against gram-positive but not against gram-negative bacteria.

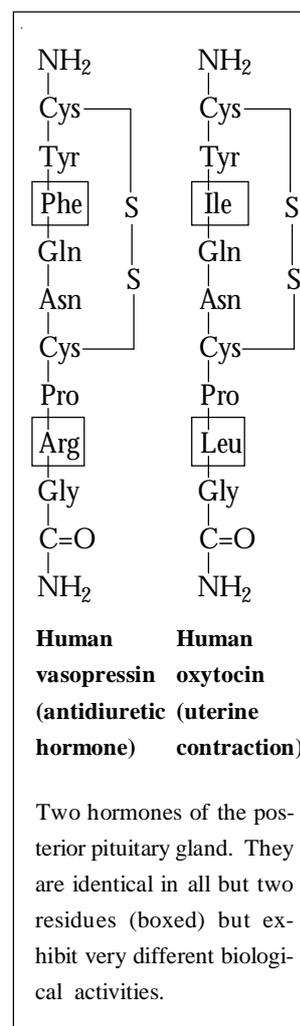
regions of the nervous system. A large number of their analogs have been synthesized to find out their role in pain transmission, since they act as transmitters of the pain-inhibitory neurons on the spinal cord. These brain morphins have been well studied which is known to allow analgesia to be separated from the development of addiction and dependence.

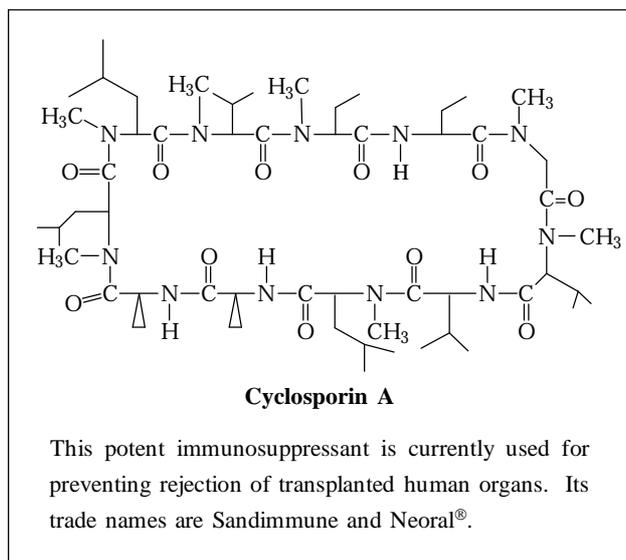
Peptide Leukotrienes: The peptide leukotrienes cause contraction of the bronchial smooth muscle and probably play an important role as mediators in allergic reactions (eg. asthma) and inflammations.

Peptide Antibiotics: The peptidic nature of penicillins has long been recognised. Penicillins and cephalosporins inhibit final stages of the enzymatic construction of the bacterial peptidoglycan cell wall component, a network of peptides and polysaccharides. The depsipeptide, valinomycin has been found to be active against a number of bacteria, yeasts and fungi.

Peptides of Immunological Significance: Immune responses to synthetic peptides constitute another important field of study. Serums raised against synthetic peptides are highly specific reagents for the corresponding native proteins from which the peptide amino acid sequence was derived. These antipeptide serums are powerful reagents for the detection and characterization of proteins.

Poisonous Peptides: The most important component of snake venoms are polypeptide neurotoxins such as cobrotoxin, which produce lethal flaccid paralysis through a neuromuscle block. The peptides of amanita mushrooms such as antamanides are



**Box 5.**

What of the future? There would be a fusion of biotechnological and chemical synthetic approaches to peptide and protein manufacture resulting in synthetic peptide vaccines.

among the best known peptide poisons. The smallest dose of 50 mg can kill a 20 gm mouse within a few hours (2.5 mg per kg of body weight). A search for inhibitors or antidotes through antagonists can be predicated.

Others: Actinomycin belongs to a class of chromopeptides and is characterized by its cytostatic growth inhibition in tumors and for antibacterial action. The cyclic desipeptides, destruxins act as insecticides whereas bialaphos (phosphinotri-cylalanyl alanine) has a strong herbicidal action. Peptides can be either sweet or bitter. The bitter peptide Arg-Gly-Pro-Pro-Ile-Val, isolated from casein hydrolysates and delicious tasting peptides from fish proteins has gained practical importance in the food industry. Nisin, produced by *Streptococcus lactis* is used as a food preservative. The atrial natriuretic peptides have diuretic properties. The phosphopeptide, (*S*)-alanyl-(*R*)-1-aminoethyl-phosphoric acid (alafosfalin) is effective against both gram positive and gram negative microorganisms. Albomycins are nucleopeptides which possess iron-complexing properties. Several muramyl peptides (glycopeptides) have the possibility of being used as adjuvants in combination with vaccines or with antibiotics.



Concluding Remarks

The ever increasing body of knowledge in peptides clearly shows that the variety of them synthesized or isolated from natural sources is of tremendous significance. Due to the denaturation and enzymatic degradation of peptide substances in the gastrointestinal tract, they cannot be taken orally, and at present, they find clinical use as parenteral, sublingual or intranasal administration only. Introduction of a wider range of active peptide substances in pharmacotherapy will have to wait to foresee the development of peptide drugs that are administered orally.

Acknowledgements

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Suggested Reading

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