# Peptide Based Biological Active Molecules

# Peptid Temelli Biyolojik Aktif Moleküller

Review Article

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**ABSTRACT**

There has been an enormous development in peptide chemistry with regard not only to the isolation, synthesis, structure identification, and elucidation of the mode of action of peptides, but also to their applications as tools in the life sciences. Peptides have been of interest in biochemistry, chemistry, biology, pharmacology, medicinal chemistry, biotechnology, and gene technology. These important natural products have a broad range with in point of their complexity. Since different amino acids are connected via peptide bonds to produce a peptide or a protein, a number of different sequences are possible–depending on the different building blocks and length of the peptide. As all peptides display a high degree of conformational diversity, it follows that many diverse and highly specific structures can be observed. Although many researches have dealt with the synthetic aspects of peptide chemistry, this review covers its biological applications of peptide based molecules.

**Key Words**
Peptide, hormones, neuropeptides, antimicrobial, antibiotics, dendrimer.

**ÖZET**

Peptit kimyasındaki muazzam gelişmeler sadece izolasyon, sentez, yapı tanımlama ve peptidlerin eylem şeklinin aydınlatılması açısından olmamış, aynı zamanda yaşam bilimleri araçları olarak uygulamalarında da büyük gelişmeler olmuştur. Peptitlere, biyokimya, kimya, biyoloji, farmakoloji, tıbbi kimya, biyoteknoloji ve gen teknolojisi alanlarının da ilgileri vardır. Bu önemli doğal ürünlerin karmaşık yapıları nedeniyle geniş bir yelpazesi vardır. Farklı amino asitler, peptid bağı ile birleşerek peptid veya proteinleri oluştururlar. Böylelikle farklı yapı blokları ve uzunluğa bağlı olarak farklı dizilerin oluşması mümkündür. Tüm peptidler yüksek derecede konformasyonel çeşitlilik gösterirler. Buna bağlı olarak pek çok farklı ve çok özel yapılar gözlenebilir. Pek çok araştırma, sentetik peptid kimyası alanıyla ilgili olmasına rağmen, bu derleme peptid bazlı moleküllerin biyolojik uygulamalarını kapsar.

**Anahtar kelimeler**
Peptid, hormonlar, nöropeptidler, antimikrobiyal, antibiyotikler, dendrimer.

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## INTRODUCTION

Peptides are amino acids polymers, connected by amide bonds (peptide bonds) between the carboxy group of one building block and the amino group of the following block. In addition to linear peptides, there are cyclic peptides, macrocycles composed of amino acids, which occur in different ring sizes. Formally, cyclic peptides are formed upon formation of a peptide bond between the amino and carboxy termini of a linear peptide (Figure 1) [1].

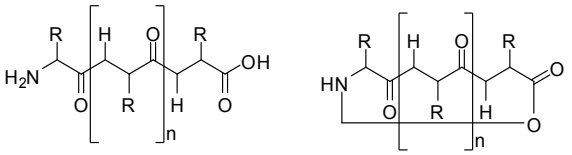



**Figure 1.** General structure of normal and cyclic peptide.

Peptide research has gained considerable interest during the past few decades. The progress in this important discipline of natural product chemistry is reflected in a flood of scientific data. Many important physiological and biochemical functions of life are affected by peptides. Peptides function as neurotransmitters, neuromodulators, and hormones in receptor-mediated signal transduction. They influence cell-cell communication upon interactions with receptors, and are involved in a number of biochemical processes, such as metabolism, pain, reproduction, and immune response [1].

Synthetic peptides function as antigens to raise antibodies, as enzyme substrates, or as enzyme inhibitors affecting signaling pathways in biochemical research or pathologic processes in medical research (Figure 2). Peptide ligands immobilized to a solid matrix may also facilitate specific protein purification [1]. Protein-protein interactions can be manipulated by small synthetic peptides. The "peptide dissection approach" uses relatively short peptide fragments that are part of a protein sequence. The synthetic peptides are investigated for their ability to fold independently, with the aim to improve the knowledge on protein folding [1]. Recombinant polypeptides and proteins have also contributed important progress in terms of methodology. Genetically engineered pharmaproteins verify the concept of therapy with endogenous protein drugs (endopharmaceuticals). Cardiovascular diseases, tumors, auto-immune diseases and infectious diseases are the most important indications [1].

The usage and need of synthetic peptides in biological applications are steadily increasing. The new targets do not allow for an isolated position of peptide chemistry exclusively oriented towards synthesis. Modern interdisciplinary science and research require synthesis, analysis, isolation, structure determination, conformational analysis and molecular modeling as integrated components of a cooperation between biologists, biochemists, pharmacologists, medical scientists, biophysicists, and bioinformaticians. Studies on structure-activity relationships involve a large number of synthetic peptide analogues with sequence variation and the introduction of nonproteinogenic buildings blocks. The concept of solid-phase peptide synthesis has exerted considerable impact on the life sciences, whilst methods of combinatorial peptide synthesis allow for the simultaneous creation of peptide libraries which contain at least several hundreds of different peptides. The high yields and purities enable both *in vitro* and *in vivo* screening of biological activity to be carried out. Special techniques make it possible to create peptide libraries that contain several hundred peptides which offer an interesting approach in the screening of new lead structures in pharmaceutical developments [1,2].

Peptide drugs, however, can be applied therapeutically only to a limited extent because of their chemical and enzymatic instabilities. Many peptides are inactive when applied orally, and even parenteral applications (intravenous or subcutaneous injection) are often not efficient because proteolytic degradation occurs on the locus of the application. Application via mucous membranes (e.g., nasal) is promising. Despite the utilization of special depot formulations and new applications systems, a major strategy in peptide chemistry is directed towards chemical modification in order to increase its chemical and enzymatic stability, to prolong the time of action, and to increase activity and selectivity towards the receptor [1,2].

The synthesis of analogues of bioactive peptides with unusual amino acid building blocks,

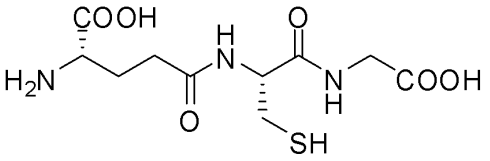



**Figure 2.** The structure of Glutathione.

linker or spacer molecules and modified peptide bonds is directed towards the development of potent agonists and antagonists of endogenous peptides. Once the amino acids of a protein that are essential for the specific biological mode of action have been revealed, these pharmacophoric groups may be incorporated into a small peptide. Rational drug design has contributed extensively in the development of protease-resistant structural variants of endogenous peptides [1,3].

Although it has been predicted that organic chemistry and biology would, in the near future, turn to peptide and protein research, interest peptide research was rather poor until the early 1940s because the number of known biologically attractive peptides was very limited at that time.

The importance and broad functional role of peptides in life processes became apparent only in the 1950s and early 1960s, when the continuous development of increasingly sensitive analytical methods and techniques for isolation and purification signaled the start of a new era in this research field. Size-exclusion chromatography [4], chromatography on cellulose-based ion-exchangers [5], counter current distribution [6] and other methods developed in various areas of biochemistry complemented the techniques for peptide isolation that had been developed previously [1]. Peptides are majorly classified in four different families:

**1. Peptide and protein hormones**

Many biochemical communications are mainly based on the action of hormones and neurotransmitters. The chemically heterogeneous group of hormones contains a variety of peptides, proteins, and amino acids. Peptide and protein hormones serve their action via binding to specific receptors (Figure 3) [7].

*Liberins and Statins*: Hormones which are releasing factors from the hypothalamus should

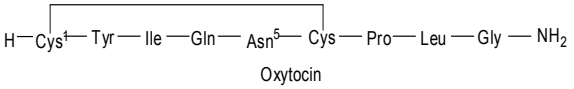



**Figure 3.** The structure of Oxytocine.

end in –liberin and which are release-inhibiting factors from the hypothalamus should end in-statin.

*Thyroliberin* (TRH) was the first peptide hormone from the hypothalamus to be isolated, structurally characterized, and synthesized [8]. TRH functions as a neurotransmitter as well as a neurohormone. Pharmacological activities of TRH associated with the CNS, such as reversal of barbiturate and ethanol hypnosis and other observations, support a neurotransmitter-like effect of TRH. The rather unique structure of TRH is resistant to digestive enzymes of the gastrointestinal tract and is thus orally active [9].

*Pituitary hormones:* The hypophysis or pituitary gland is a vertebrate endocrine gland located at the base of the brain, and connected to the midbrain by the hypophyseal stalk. The hypophysis consists of the anterior pituitary (adenohypophysis), the middle part, and the posterior pituitary (neurohypophysis). The pituitary hormones are released into the bloodstream and stimulate their corresponding glands or tissues (adrenal cortex, thyroid, testes/ovaries, liver, and other special tissues) to secrete the appropriate endocrine hormones [7].

*Somatropin*: This peptide functions as a growth hormone. The hormone, a 191 amino acid long protein, had always shown some difficulties to be analyzed [10].

*Neurohypophyseal hormones*: The neurohypophysis is the posterior lobe of the pituitary, and is anatomically distinct from the adenohypophysis. It secretes oxytocin (OT) and vasopressin (VP), two homologous heterodetic cyclic peptides that are primarily synthesized in the hypothalamus [11, 12].

Oxytocin is a mammalian nanopeptide hormone produced in the hypothalamus and secreted by the posterior pituitary gland into the circulation. It is also synthesized in the peripheral tissues of the uterus, testis, and heart. Oxytocin exhibits a range of physiological roles including mammary and uterine smooth muscle contraction, neurotransmission in

the central nervous system, and autocrine and/or paracrine functions in the ovaries and testes [13].

*Gastrointestinal hormones*: To date, more than 30 peptide hormone genes are known to be expressed throughout the digestive tract. Consequently, the gut is the largest endocrine hormone-producing organ in the body, both in terms of the number of endocrine cells and the number of hormones [14,15].

Relaxin is a small 6 kDa two-chain peptide member of the insulin superfamily that is principally produced in the corpus luteum of the ovary and which plays a key role in connective tissue remodeling during parturition. Like insulin, it is produced on the ribosome as preprohormone that undergoes oxidative folding and subsequent proteolytic processing to yield the mature insulin-like peptide. In contrast to the now considerable insight into insulin chain folding and oxidation, comparatively little is known about the folding pathway of relaxin [16].

*Pancreatic islet hormones:* Pancreatic islet hormones are responsible for regulating not only the storage of glucose and fatty acids, but also their release. Three different types of cells are found in the pancreatic islets of Langerhans, and each secretes a specific polypeptide hormone. The-cells secrete glucagon, the cells insulin, and the cells somatostatin.

Insulin is a protein hormone consisting of an A-chain (21 residues) and a B-chain (30 residues) linked by two disulfide bonds [17]. In addition, the A-chain contains an intrachain disulfide bond. In the bloodstream, insulin is present at very low concentrations. At these low concentrations, insulin is monomeric, which is its biologically active form. At higher concentrations, insulin assembles into dimers and, in the presence of zinc ions at neutral pH, into hexamers [18].

**2. Neuropeptides**

Peptides are ubiquitous bioactive compounds that occur throughout the body, and in almost every type of organisms. Several peptides previously considered to be brain peptides have also been found in the gut. Other peptides, which in the past were considered to be gut peptides are now known to be located also in the brain, and hence it is difficult to make a clear-cut definition of neuropeptides. CCK has long been known as a gastrointestinal hormone that mediates digestive functions and feeding behavior, but in 1975 it was also identified in rat brain [1].

*Opioid Peptides*: Opiates such as morphine have been widely used by clinicians both for the blockade of severe pain and for anesthesia [19]. Methionineenkephalin, (Tyr-Gly-Gly-Phe-Met), plays a role in cell proliferation and tissue organization during development, cellular renewal, wound healing, and angiogenesis, but also in cancer [20].

*Tachykinins*: The term tachykinins describes a family of peptides that shares a common C-terminal sequence (-Phe-Xaa-Gly-Leu-Met-$NH_2$). They exist both in mammalian and nonmammalian species. The tachykinins (neurokinins) are a family of closely related mammalian neuropeptides that display a variety of biological activities including pain transmission, smoothmuscle contraction, bronchoconstriction, vasodilation, activation of the immune system, and neurogenic inflammation [21].

Tachykinin actions are mediated by at least three distinct cell-surface receptors, NK1, NK2, and NK3, that show preferential binding for the endogenous agonists substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), respectively [22].

**3. Peptide Antibiotics**

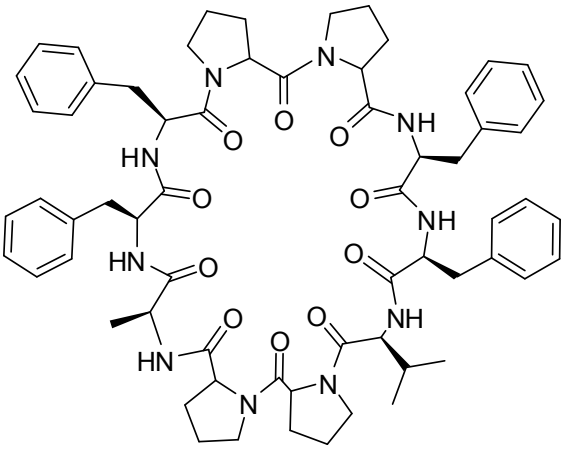
Antibiotics, derived from the Greek antibios which means "against life", are a chemically heterogeneous group of substances produced by microorganisms (bacteria and fungi) which kill or inhibit the growth of other microorganisms. Initially, peptide antibiotics can be classified according to their chemical structure into linear and cyclic compounds, which can be further subdivided into homomeric peptide antibiotics exclusively composed of amino acids, and heteromeric ones additionally containing nonamino acid-derived building blocks [23-25].

Viomycin contains the unusual noncoded amino acids tuberactidine and ureidodehydroalanine in addition to a-lysine, serine, and diaminopropionic

acid. It shows inhibitory effects on translation similar to those of aminoglycosides and is a competitive inhibitor of the group I intron, hepatitis delta virus (HDV), and hammerhead ribozymes Viomycin also induces interactions between RNA molecules [26].

**4. Peptide Toxins**

Peptide and protein toxins are mostly low-molecular weight, single-chain compounds produced by snakes and invertebrates, as well as by virulent strains of bacteria and some plants. For example, spider venoms are complex mixtures of low molecular mass organic molecules (< 1 kDa), polypeptides (3-10 kDa) and high molecular mass proteins (> 10 kDa). A vast majority of spider toxins are polypeptide toxins with 3-5 disulfide bonds which display various structures and biological activities. Spider peptide toxins have proved to be a powerful tool for the study of voltage-sensitive and ligand-gated ion channels and have potential applications as novel pharmaceutical drugs [27]. Interestingly, antamanide (Figure 4), which is a cyclic decapeptide of A. phalloides, protects experimental animals from intoxication by Amanita-derived phalloidin.



Antamanide

**Figure 4.** The structure of Antamanide.

**Drug Delivery**

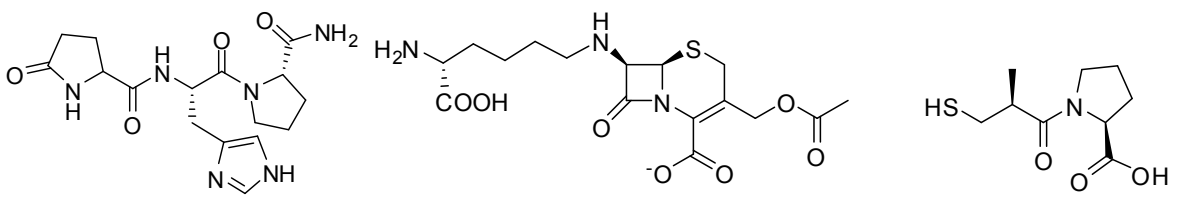
During the past decade, advances in biotechnology enable us to design more sophisticated approaches for drug delivery. For example, biological compounds such as peptides, proteins, and antibodies are employed to target angiogenesis [28]. Nanoparticles are proposed for passive targeting of tumors [29]. Traditional materials used for drug delivery are generally controlled and degraded by chemical reactions such as spontaneous hydrolysis of ester linkages. Biomaterials that are responsive to physiological stimulus and at the same time release an adaptable dosage may reduce treatment failure from pharmacogenomic differences of individual patients (Figure 5) [30,31].

There is an urgent interest how certain peptides interact with and translocate through biological membranes. The peptide "penetratin", corresponding to the 16-mer third helix of the homeodomain of the Antennapedia protein, has been reported to permeate eukaryotic cell membranes and may be used as a carrier to transport oligonucleotides, peptide nucleic acids, and small proteins into live cells [28-32].

Initially, use of protein and peptides as pharmaceuticals was severely limited, as they were difficult to produce and were isolated from animal sources. These protein and peptide products obtained from animals differed from functional molecules present in the human body, and their use as therapeutic agents raised concerns with regard to their immunogenic potential [33,34].

**Peptide Dendrimers**

Peptide dendrimers can generally be defined as macromolecules that contain peptide bonds in their structure. The biocompatibility and



Thyroliberin Cephalosporin C Captopril

**Figure 5.** The structures of Thyroliberin (a), Cephalosporin C (b), and Captopril (c).

immunocompatibility of polymeric materials are mandatory for their therapeutic utility [35]. Some potential polymer therapeutics have been, or are being, withdrawn from the market due to adverse side effects. Polymer therapeutics is a term used to describe polymeric drugs, polymer-drug conjugates, polymer-protein conjugates, polymer micelles to which drugs are covalently bound, and multicomponent polyplexes that are being developed as nonviral vectors [36,37].

**Antimicrobial Peptides**

They are cationic and amphipathic peptides that bind to the membrane surface, adopting a predominantly R-helical structure. At high concentrations they permeabilize the lipid matrix, forming waterfilled, nanometer-sized pores that lead to cell death. Experimentally, it is observed that as the concentration of bound peptide is increased past the permeabilization threshold there is a change in the orientation from the peptides being essentially parallel.

The peptides are believed to stabilize pores by interacting strongly with the lipid headgroups that line pores. In fact, despite being very intensively studied experimentally, relatively little is known regarding the mechanism of pore formation or the structure of the pore itself. For example MG-H2 (IIKKFLHSIWKFGKAFVGEIMNI) is positively charged (+3) at physiological pH [38].

**Enzyme Inhibitor Peptides**

These type of peptides bind enzyme active sites and block it. In some issues peptides replace the enzyme. For example, PQQGDH replaced glucose oxidase (GOD) as the major enzyme used in glucose sensor systems. Mainly due to its high catalytic activity and non-dependence on oxygen as an electron acceptor [39]. The usage of peptides as enzyme inhibitors or activators will be promising for pharmacological and medicinal chemistry studies.

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