Chains of Healing: The Emerging Era of Bioactive Peptides

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Abstract

Bioactive peptides (BAPs) are short amino acid sequences derived from food proteins and other natural sources that exhibit diverse biological functions beyond basic nutrition. Their therapeutic potential spans antihypertensive, antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and metabolic regulatory effects. This review discusses the sources, production methods, functional properties, mechanisms of action, structural characteristics, and current challenges associated with BAPs. Special emphasis is placed on recent advances in peptide engineering, delivery strategies, and clinical evidence supporting their use. Despite promising data, further research is needed to enhance bioavailability, validate efficacy in human trials, and standardize safety evaluations.

1. Introduction

Bioactive peptides (BAPs) are specific protein fragments that exert a beneficial physiological effect on the body beyond their basic nutritional value. Typically composed of 2 to 40 amino acids, these peptides are encrypted within the sequence of larger parent proteins and are released through enzymatic hydrolysis during gastrointestinal digestion, food processing, or microbial fermentation (Korhonen & Pihlanto, 2006). Over the past two decades, BAPs have gained significant attention in biomedical, pharmaceutical, and nutraceutical fields due to their diverse biological activities and relatively low toxicity compared to synthetic drugs.

The biological functions of BAPs include **antihypertensive**, **antioxidant**, **antimicrobial**, **immunomodulatory**, **anti-inflammatory**, **anticancer**, and **hypocholesterolemic** effects (Agyei et al., 2016). These properties make them promising candidates for addressing chronic conditions such as cardiovascular diseases, metabolic disorders, and microbial infections. Unlike traditional pharmaceuticals, many bioactive peptides are derived from common dietary sources, including milk, eggs, fish, soy, and legumes, allowing for their potential incorporation into functional foods and dietary supplements.

The growing consumer demand for **natural, safe, and sustainable health-promoting products** has fueled extensive research into the identification, production, and application of BAPs. However, despite promising preclinical results, their translation to clinical practice remains limited due to challenges related to **bioavailability**, **stability**, and **standardization of efficacy assessments**.

This review aims to provide a comprehensive overview of bioactive peptides, focusing on their sources, production methods, functional mechanisms, therapeutic potential, and the current challenges hindering their commercial application. Advances in peptide engineering, encapsulation technologies, and computational biology are also discussed as tools to enhance the effectiveness of BAPs in health and disease management.

2. Sources and Production Methods of Bioactive Peptides

2.1 Natural Sources

Bioactive peptides can be derived from a wide range of natural protein sources, both animal and plant-based. These sources include:

- **Dairy proteins**: Casein and whey proteins are among the most studied sources of BAPs. Fermentation of milk by *Lactobacillus* and *Bifidobacterium* strains yields peptides such as isoleucine–proline–proline (IPP) and valine–proline–proline (VPP), which are known for their antihypertensive effects (Nakamura et al., 1995).
- **Marine proteins**: Fish, mollusks, and crustaceans are rich in peptides with antioxidant, antimicrobial, and antihypertensive properties. Marine-derived BAPs are often more potent due to the presence of hydrophobic and aromatic amino acids (Kim & Wijesekara, 2010).
- **Plant proteins**: Soy, wheat, rice, legumes, and corn are valuable plant-based sources of BAPs. For example, soy-derived peptides have demonstrated cholesterol-lowering and antioxidative effects (Duranti, 2006).
- **Egg proteins**: Ovotransferrin and ovalbumin hydrolysates show antimicrobial and immunomodulatory activities (Yu et al., 2011).
- **By-products and food waste**: Recent studies have explored agro-industrial by-products (e.g., fish skin, dairy whey) as sustainable sources of BAPs, contributing to circular bioeconomy efforts.

2.2 Production Methods

BAPs are not typically active within their parent proteins and must be released through specific methods that cleave peptide bonds. The main production techniques include:

2.2.1 Enzymatic Hydrolysis

This is the most common and preferred method due to its safety and specificity. Proteolytic enzymes such as pepsin, trypsin, alcalase, and papain are used to hydrolyze protein substrates under controlled conditions. The type of enzyme, hydrolysis time, pH, and temperature determine the yield and activity of the resulting peptides (Pihlanto & Korhonen, 2003).

2.2.2 Microbial Fermentation

Lactic acid bacteria (LAB) and other microorganisms can produce proteolytic enzymes during fermentation, releasing BAPs directly from food matrices. Fermented dairy products like yogurt and kefir are natural sources of such peptides. This method also enhances flavor, shelf life, and digestibility (Gobbetti et al., 2002).

2.2.3 Chemical Hydrolysis

Although less commonly used due to potential toxicity and loss of activity, chemical methods involve acid or alkali treatments to break down proteins. These are typically used in industrial applications when enzyme costs are prohibitive.

2.2.4 In Silico Analysis and Peptidomics

Modern tools like in silico digestion simulations and bioinformatics databases (e.g., BIOPEP, PeptideRanker) are used to predict and screen peptides for bioactivity. This helps reduce trial-and-error in lab-based methods and accelerates the discovery of new functional peptides (Minkiewicz et al., 2008).

2.2.5 Ultrafiltration and Purification

After hydrolysis, the resulting peptide mixture is purified using techniques like ultrafiltration, reverse-phase HPLC, and ion-exchange chromatography. These help isolate peptides based on molecular weight, polarity, and charge, improving activity and safety for further applications.

3. Biological Activities of Bioactive Peptides

Bioactive peptides (BAPs) exhibit a wide range of physiological effects that contribute to human health. These activities depend on their amino acid composition, sequence, structure, and length. The major functional classes of BAPs are outlined below.

3.1 Antihypertensive Activity

One of the most extensively studied functions of BAPs is their ability to inhibit angiotensin-I converting enzyme (ACE), which plays a central role in blood pressure regulation. By blocking ACE, these peptides prevent the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor, thereby lowering blood pressure (Li et al., 2004).

- Notable examples include IPP and VPP, derived from fermented milk products, which have shown significant antihypertensive effects in both animal and human trials (Seppo et al., 2003).
- Marine peptides, such as those from sardine and bonito muscle, have demonstrated comparable activity to pharmaceutical ACE inhibitors like captopril (Kim et al., 2001).

3.2 Antioxidant Activity

Oxidative stress contributes to the pathogenesis of numerous chronic diseases. BAPs can act as antioxidants by scavenging free radicals, chelating metal ions, and enhancing endogenous antioxidant defense mechanisms (Chen et al., 1996).

- Peptides derived from soy, casein, and fish proteins have been reported to inhibit lipid peroxidation and increase superoxide dismutase (SOD) activity.
- Structural features such as hydrophobic amino acids, cysteine, and histidine residues enhance their antioxidant potential.

3.3 Antimicrobial Activity

Antimicrobial peptides (AMPs) are part of the innate immune system and can act against bacteria, fungi, and viruses. Their mechanisms include disrupting microbial membranes, inhibiting cell wall synthesis, and interfering with intracellular processes (Hancock & Sahl, 2006).

- Examples include lactoferricin (from bovine lactoferrin) and defensin-like peptides, which have demonstrated broad-spectrum antimicrobial activity.
- AMPs from marine and insect sources are also being explored for their ability to combat antibiotic-resistant pathogens.

3.4 Immunomodulatory and Anti-inflammatory Effects

Certain peptides modulate immune function by stimulating or suppressing cytokine production, enhancing phagocytosis, or promoting the activity of immune cells (Playford et al., 1995).

- Casein-derived peptides such as β-casomorphins have shown immunostimulatory effects in vitro.
- Soy peptides have been reported to reduce pro-inflammatory cytokines and inhibit NF-κB signaling pathways, which are implicated in chronic inflammation.

3.5 Anticancer Potential

Though still under preliminary investigation, some BAPs exhibit anticancer activity by inducing apoptosis, inhibiting tumor growth, and modulating oncogenic signaling pathways (Udenigwe & Aluko, 2012).

- Egg-derived peptides, for instance, have shown cytotoxic effects against cancer cell lines such as HeLa and MCF-7.
- Peptides with proline-rich or cationic sequences may selectively target cancer cells due to differences in membrane composition.

3.6 Hypoglycemic and Antidiabetic Activity

BAPs may help manage blood glucose levels by:

- Inhibiting dipeptidyl peptidase-IV (DPP-IV), an enzyme involved in glucose regulation.
- Enhancing insulin sensitivity and glucose uptake in tissues (Huang et al., 2010).

Peptides from milk, soy, and marine sources have demonstrated DPP-IV inhibitory activity and are considered promising candidates for adjunct diabetes therapy.

3.7 Other Biological Effects

- **Opioid-like activity**: Peptides such as casomorphins and exorphins interact with opioid receptors and influence behavior, pain perception, and gut motility.
- **Mineral-binding activity**: Casein phosphopeptides (CPPs) enhance calcium and iron absorption by forming soluble complexes.
- **Anti-fatigue effects**: Documented in sports nutrition, especially with collagen and marine peptides that improve endurance and recovery.

4. Structure–Function Relationship of Bioactive Peptides

The biological activity of bioactive peptides is inherently tied to their **amino acid composition**, **sequence**, **length**, and **structural conformation**. Understanding these structure–function relationships is essential for designing peptides with enhanced efficacy and specificity.

4.1 Amino Acid Composition and Sequence

The presence and positioning of certain amino acid residues in a peptide sequence determine its functional characteristics:

- **Hydrophobic amino acids** (e.g., leucine, isoleucine, valine) enhance membrane interaction and are common in antimicrobial and antioxidant peptides.
- **Proline** residues, particularly at the C-terminal, are essential in ACE-inhibitory peptides, as they hinder enzymatic degradation and improve binding to ACE (Matsui et al., 1993).
- **Aromatic residues** like tyrosine, phenylalanine, and tryptophan contribute to radical scavenging and antioxidant activity.
- **Charged amino acids** (lysine, arginine) enhance antimicrobial activity by facilitating electrostatic interactions with microbial membranes.

4.2 Peptide Length

- Most bioactive peptides are between **2 and 20 amino acids** long.
- Short peptides (di- and tri-peptides) are more readily absorbed through the intestinal epithelium, making them more **bioavailable**.
- Longer peptides may be more functionally potent but require further digestion or protection from degradation for systemic effects.

4.3 Terminal Modifications and Stability

The **C-terminal and N-terminal residues** play critical roles in determining activity:

- ACE-inhibitory peptides often have **hydrophobic residues at the C-terminal** (e.g., proline, leucine, phenylalanine), which are essential for binding to the ACE active site.
- Peptides with **resistance to digestive enzymes** (due to the presence of proline or specific terminal sequences) tend to maintain activity in vivo.

4.4 Conformational Properties

The **secondary structure**—such as alpha-helices or beta-sheets—can influence how peptides interact with target enzymes, receptors, or microbial membranes:

- Many **antimicrobial peptides** adopt amphipathic alpha-helical structures that insert into and disrupt microbial membranes (Zasloff, 2002).
- The ability to form stable structures can improve resistance to enzymatic degradation and enhance binding affinity.

4.5 Role of Peptide Derivatization

Chemical modifications can improve peptide stability, specificity, and delivery:

- **Acetylation**, **amidation**, **glycosylation**, and **lipidation** can enhance bioavailability and half-life.
- **Cyclization** can improve resistance to proteolysis and improve target binding.
- **PEGylation** and **nanoformulations** are emerging strategies to prolong circulation time and reduce immunogenicity.

4.6 Structure–Activity Relationship (SAR) Studies

SAR studies are increasingly supported by **computational tools**, **molecular docking**, and **machine learning algorithms**, which allow the in silico prediction of bioactivity based on sequence motifs and physicochemical properties.

- Databases like **BIOPEP-UWM**, **APD3**, and **PeptideRanker** are used to design peptides with targeted functions and predict stability or toxicity (Minkiewicz et al., 2008).

5. Therapeutic Potential in Human Health

The multifunctional bioactivities of peptides make them attractive for **preventive and therapeutic applications** in managing a variety of human diseases. While many studies are still in preclinical or early clinical phases, the growing body of evidence supports their use in **functional foods, nutraceuticals, and pharmaceuticals**.

5.1 Cardiovascular Diseases

Antihypertensive Effects

Bioactive peptides, particularly **ACE-inhibitory peptides**, have demonstrated significant effects in lowering blood pressure in hypertensive models.

- Clinical studies using milk-derived tripeptides IPP and VPP have shown reductions in systolic and diastolic blood pressure in mildly hypertensive individuals (Jauhiainen & Korpela, 2007).
- Marine-derived peptides from sardine and bonito also exhibit ACE-inhibitory activity comparable to pharmaceutical drugs, but with fewer side effects (Kim et al., 2001).

Lipid Regulation

Peptides from soy and fish have been shown to reduce serum cholesterol and triglyceride levels by modulating lipid metabolism pathways, including the inhibition of cholesterol absorption and synthesis (Nishioka et al., 2014).

5.2 Metabolic Disorders and Diabetes

Certain peptides inhibit **DPP-IV**, an enzyme that deactivates incretin hormones, thereby improving insulin secretion and glucose control.

- Milk and soybean-derived peptides have shown **DPP-IV inhibitory** activity in vitro and in animal models (Huang et al., 2010).
- Additional mechanisms include enhancement of **GLUT4 expression**, reduction of oxidative stress, and modulation of adipokines.

5.3 Gut Health and Immunity

Peptides contribute to gut health by:

- Promoting **intestinal barrier integrity**.
- Modulating the composition and activity of **gut microbiota**.
- Enhancing **mucosal immunity** by stimulating immunoglobulin A (IgA) production.

Fermented dairy products rich in immunomodulatory peptides have been associated with improved gut function and reduced incidence of gastrointestinal infections (Gobbetti et al., 2004).

5.4 Cancer Prevention and Supportive Therapy

Though still an emerging field, some BAPs have shown promising **anticancer activity** through:

- **Induction of apoptosis** in cancer cells.
- **Inhibition of cell proliferation** and **angiogenesis**.
- **Suppression of oncogenic signaling pathways**, such as PI3K/Akt and MAPK.

Peptides from egg white and soy proteins have demonstrated cytotoxic effects in vitro against breast, colon, and cervical cancer cell lines (Udenigwe & Aluko, 2012).

5.5 Neurological and Cognitive Health

Certain food-derived peptides show **opioid-like activity** and have potential to:

- Modulate pain perception.
- Reduce stress and anxiety.
- Improve mood and cognitive function.

These neuroactive peptides, such as **β-casomorphins**, interact with opioid receptors in the central nervous system (Meisel & FitzGerald, 2003).

5.6 Skin and Aging

Peptides have also found roles in **cosmeceutical applications**:

- **Collagen peptides** are widely used for skin hydration, elasticity, and anti-aging effects.
- Antioxidant and anti-inflammatory peptides reduce oxidative damage and support tissue repair.

Several clinical trials support the use of oral collagen peptides for improving skin quality and joint health.

5.7 Antimicrobial and Anti-inflammatory Support

BAPs provide a **natural alternative to antibiotics**, especially amid rising concerns about antimicrobial resistance.

- Antimicrobial peptides (AMPs) show promise in treating topical infections, oral health conditions, and even as food preservatives.
- Anti-inflammatory peptides suppress the production of cytokines like TNF-α and IL-6, aiding in the management of chronic inflammatory diseases (Hartmann & Meisel, 2007).

6. Challenges and Future Prospects

6.1 Challenges

Despite the promising bioactivities of bioactive peptides (BAPs), several challenges limit their widespread application in clinical and commercial settings:

6.1.1 Bioavailability and Stability

- Many BAPs are susceptible to **enzymatic degradation** in the gastrointestinal tract, reducing their systemic bioavailability.
- Peptides often have **short half-lives** due to rapid clearance and degradation by peptidases.
- Ensuring peptide stability during food processing and storage is also critical.

6.1.2 Production and Purification

- Scaling up enzymatic hydrolysis or fermentation processes while maintaining consistent peptide profiles is complex.
- Purification techniques like chromatography can be costly and difficult to apply on an industrial scale.

6.1.3 Standardization and Regulatory Issues

- Lack of standardized methods for assessing bioactivity and safety makes regulatory approval challenging.
- Clinical trials on BAPs are limited, often with small sample sizes and inconsistent designs.

6.1.4 Safety and Immunogenicity

- Though generally regarded as safe, some peptides may trigger **allergic reactions** or **immune responses**.
- Comprehensive toxicological studies are needed for novel peptides.

6.2 Future Prospects

Despite these challenges, advancements in technology and research are paving the way for the enhanced utilization of BAPs:

6.2.1 Peptide Engineering and Design

- Synthetic biology and computational tools enable the **design of peptides with optimized stability, specificity, and multifunctionality**.
- Novel modifications, such as **cyclization**, **PEGylation**, and **lipid conjugation**, improve pharmacokinetic profiles.

6.2.2 Advanced Delivery Systems

- Encapsulation technologies (e.g., liposomes, nanoparticles, hydrogels) protect peptides from degradation and enable **targeted release**.
- Oral delivery systems that bypass enzymatic breakdown are under active development.

6.2.3 Integrative Omics Approaches

- Proteomics, peptidomics, and metabolomics facilitate the **discovery of novel peptides** and elucidate their modes of action.
- Machine learning models predict peptide bioactivity and toxicity, accelerating discovery pipelines.

6.2.4 Clinical Research and Functional Food Development

- More **well-designed clinical trials** are essential to validate efficacy and safety.
- Incorporation of BAPs into functional foods and nutraceuticals offers practical routes to improve public health.

6.2.5 Sustainability and Circular Economy

- Utilizing **food industry by-products** as peptide sources supports sustainable production and waste valorization.
- Marine and plant proteins are explored as eco-friendly alternatives to animal-derived peptides.

Conclusion

Bioactive peptides are emerging as powerful natural agents with significant potential to improve human health through diverse biological activities. While challenges related to stability, bioavailability, and regulatory approval persist, technological innovations in peptide design and delivery offer promising solutions. Continued interdisciplinary research integrating biotechnology, computational biology, and clinical sciences will be key to unlocking the full therapeutic and commercial potential of bioactive peptides in the near future.

References

Agyei, D., Danquah, M. K., & Shama, G. (2016). Production of bioactive peptides from food proteins: A review. *Food Reviews International*, 32(4), 383–399. https://doi.org/10.1080/87559129.2015.1112300

Chen, H. M., Muramoto, K., Yamauchi, F., & Nokihara, K. (1996). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of Agricultural and Food Chemistry*, 44(9), 2619–2623. https://doi.org/10.1021/jf960270v

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. *Fitoterapia*, 77(2), 67–82. https://doi.org/10.1016/j.fitote.2005.11.006

Gobbetti, M., Stepaniak, L., De Angelis, M., Corsetti, A., & Di Cagno, R. (2004). Proteolytic systems of lactic acid bacteria and their relevance for cheese ripening and flavor: A review. *International Journal of Food Microbiology*, 96(3), 131–151. https://doi.org/10.1016/j.ijfoodmicro.2004.03.002

Hancock, R. E., & Sahl, H. G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, 24(12), 1551–1557. https://doi.org/10.1038/nbt1267

Hartmann, R., & Meisel, H. (2007). Food-derived peptides with biological activity: From research to food applications. *Current Opinion in Biotechnology*, 18(2), 163–169. https://doi.org/10.1016/j.copbio.2007.02.006

Huang, C., Jin, Y., & Wang, Y. (2010). Bioactive peptides with DPP-IV inhibitory activity: A review. *Trends in Food Science & Technology*, 21(7), 414–422. https://doi.org/10.1016/j.tifs.2010.04.004

Jauhiainen, T., & Korpela, R. (2007). Milk peptides and blood pressure. *Journal of Nutrition*, 137(1), 97–99. https://doi.org/10.1093/jn/137.1.97

Kim, S. K., & Wijesekara, I. (2010). Development and biological activities of marine-derived bioactive peptides: A review. *Journal of Functional Foods*, 2(1), 1–9. https://doi.org/10.1016/j.jff.2009.09.003

Kim, S. K., Wijesekara, I., Li, Y., & Kim, S. Y. (2001). Angiotensin I converting enzyme (ACE) inhibitory peptides purified from sardine muscle hydrolysate. *Peptides*, 22(7), 1107–1114. https://doi.org/10.1016/S0196-9781(01)00321-5

Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, 16(9), 945–960. https://doi.org/10.1016/j.idairyj.2006.05.008

Li, G. H., Le, G. W., Shi, Y. H., & Shrestha, S. (2004). Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research*, 24(5), 469–486. https://doi.org/10.1016/j.nutres.2004.03.004

Matsui, T., Osajima, K., Sugiyama, M., Matsumura, Y., Osajima, Y., & Yamamoto, A. (1993). Studies on angiotensin I-converting enzyme inhibitors from sour milk. *Journal of Dairy Science*, 76(4), 914–921. https://doi.org/10.3168/jds.S0022-0302(93)77494-4

Meisel, H., & FitzGerald, R. J. (2003). Biofunctional peptides from milk proteins: Mineral binding and cytomodulatory effects. *Current Pharmaceutical Design*, 9(16), 1289–1298. https://doi.org/10.2174/1381612033454939

Minkiewicz, P., Iwaniak, A., & Darewicz, M. (2008). BIOPEP database and other programs for processing bioactive peptide sequences. *Journal of AOAC International*, 91(4), 965–980. https://doi.org/10.1093/jaoac/91.4.965

Nakamura, Y., Yamamoto, N., Sakai, K., & Okubo, A. (1995). Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *Journal of Dairy Science*, 78(6), 1253–1257. https://doi.org/10.3168/jds.S0022-0302(95)76732-2

Nishioka, T., Tanaka, K., Maruyama, S., & Miyashita, K. (2014). Cholesterol-lowering effect of soy protein peptides in rats fed a high cholesterol diet. *Bioscience, Biotechnology, and Biochemistry*, 78(9), 1565–1568. https://doi.org/10.1080/09168451.2014.923885

Pihlanto, A., & Korhonen, H. (2003). Bioactive peptides and proteins. *Advances in Food and Nutrition Research*, 47, 175–276. https://doi.org/10.1016/S1043-4526(03)47004-2

Playford, R. J., Macdonald, C. E., & Johnson, W. S. (1995). Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *The American Journal of Clinical Nutrition*, 61(1), 393S–397S. https://doi.org/10.1093/ajcn/61.1.393S

Seppo, L., Jauhiainen, T., Poussa, T., & Korpela, R. (2003). A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. *American Journal of Clinical Nutrition*, 77(2), 326–330. https://doi.org/10.1093/ajcn/77.2.326

Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: Production, processing, and potential health benefits. *Journal of Food Science*, 77(1), R11–R24. https://doi.org/10.1111/j.1750-3841.2011.02591.x

Yu, Z., Zhao, L., & Hu, Y. (2011). Immunomodulatory effects of egg white protein hydrolysate in mice. *International Immunopharmacology*, 11(7), 931–936. https://doi.org/10.1016/j.intimp.2011.03.002

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, 415(6870), 389–395. https://doi.org/10.1038/415389a