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Critical review

Pharmaceutical applications of bioactive peptides

MK Danquah[1,2]*,D Agyei[2]

Abstract

Introduction

There is a mounting interest in the therapeutic potential of bioactive peptides which collectively present a cornucopia of bioactivities for exploitation in vivo. Bioactive peptides trigger certain functionalities such as antioxidative, antimicrobial, antihypertensive, cytomodulatory and immunomodulatory activities in the living body system. With research and development, there exists an opportunity to effectively harness these functionalities for the treatment, prevention and mitigation of different medical conditions. This critical review discusses some potential therapeutic applications of bioactive peptides in the light of advances in general biopharmaceutical production based on proteomics and genomics.

Conclusion

Bioactive peptides are ubiquitous biomolecules widely abundant and easily obtained from food proteins and there is no limit to how many can be obtained froma single food protein.

Introduction

An ancient Chinese saying states that *Medicine and food have a common origin*[1]. Centuries of research has confirmed and enhanced our understanding of the intimate link between nutrition and health. Assertions about the ability of foods and its components to enhance the overall quality of life continues to captivate us even today[2]. Research has uncovered several bioactive components from food derived macro-biomolecules (such as lipids and proteins); however, protein derived biomolecules are the most diverse and most studied. Within the human body, some protein hydrolysates have been shown to trigger certain 'hormone-like' responses that offer health benefits *in vivo* and/or *in vitro*, eith-er in the intact form or as hydrolysates[3]. These protein hydrolysates, (bioactive peptides), are specific protein fragments with the ability to impart a physiologically measurable biological effect resulting in a positive impact on body functions/conditions, thus ultimately influencing health[4–6]. Some positive physiological responses of bioactive peptides are antioxidative, antimicrobial, antihypertensive, cytomodulatory and immunomodulatory effects under *in vivo* and *in vitro* conditions[3,5,7–9]. These are observed in the major body systems such as the digestive, cardiovascular, nervous, musculoskeletal and immune systems[6]. Thus, bioactive peptides can play a significant role in the pharmaceutical industry since the physiological responses induced are the same as the targeted responses intended by small molecules and biologics. This critical review highlights different therapeutic applications of bioactive peptides with recent findings in the field.

Peptides as active pharmaceutical ingredients

Proteins and peptides play important roles in living body systems by controlling and coordinating inter and intra-cellular communications and cellular function. From a nutritional perspective, peptides are more bioavailable than proteins or free amino acids[10]. Further, peptides with low molecular weight have been known to be less allergenic than their native proteins, explaining why milk protein hydrolysates are widely utilized in the formulation of hypoallergenic infant foods[11]. Additionally, as nature's tool kit, the diverse physiological roles of peptidesmake them suitable candidates for the development of therapeutic agents[12,13]. There is a wide variety of physiological activities induced by bioactive peptides and these bioactivities are determined by the type, number, sequence and properties of amino acids present in the peptide[13,14]. It is worth mentioning that whereas some proteins (such as lysozyme and α-lactalbumin) retain their bioactivities even the unhydrolysed denatured state[14], usually, the aforementioned bioactivities are latent until proteins are hydrolysed to release physiologically active peptides. Captured in Table 1 are some bioactive peptides and their bioactivities or areas of therapeutic applications. Bioactive peptides are therefore suitable candidates for a new era of pharmaceutical products, especially with the heightened concerns of side effects of small molecule drugs and the increased attention to fresher and 'greener' foods and nutraceuticals possessing health-preventing or health-promoting properties.

Changing trends

Research continues to uncover novel peptide sequences with potential applications in the prevention and mitigation of ill health, the findings of which are of tremendous significance to the scientific community, pharmaceutical corporations and consumers alike. There is also a growing interest in the level of peptide therapeutics in the

* Corresponding author
Email: mkdanquah@curtin.edu.my

[1] Department of Chemical Engineering, Curtin University of Technology, Sarawak, Malaysia
[2] Bio Engineering Laboratory, Chemical Engineering Department, Monash University, Australia

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Table 1 Some food protein hydrolysates and their therapeutic application

Protein source	Microorganism or enzyme used	Amino acid sequence	Therapeutic application (bioactivity)*	Reference
Caprine milk	Pepsin	α_{s2}-casein f(203–208)	Antimicrobial; antihypertensive; antioxidant	Atanasova and Ivanova[19]
Bovine milk	*Lb. helveticus*	Ile-Pro-Pro; Val-Pro-Pro	Antihypertensive*	Jäkälä and Vapaatalo[38]
Bovine milk	Porcine intestinal enzymes	(Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn-Ser-Leu) β-casein f(60–70)	Immunostimulatory, opioid agonist; ACE-inhibitory	Atanasova and Ivanova[19]; Meisel[59]
Bovine milk	*Enterococcus faecalis* TH563 and *Lb. delbrueckii* ssp. *bulgaricus* LA2	Unidentified	Immunostimulatory, ACE-inhibitory	Regazzo *et al.*[60]
Egg white	Alcalase	Arg-Val-Pro-Ser-Leu	ACE-inhibitory	Liu *et al.*[58]
Rice endosperm	Neutrase	Phe-Arg-Asp-Glu-His-Lys-Lys; and Lys-His-Asp-Arg-Gly-Asp-Glu-Phe	Antioxidative	Zhang *et al.*[61]
Sweet potato juice	Thermoase PC10F, Protease S and Proleather FG-F	Ile-Thr-Pro; Ile-Ile-Pro; Gly-Gln-Tyr; Ser-Thr-Tyr-Gln-Thr	ACE-inhibitory	Ishiguro *et al.*[62]

Lb., *Lactobacillus;* ACE, angiotensin converting enzyme.
*Meta-analyses of clinical studies.

pharmaceutical industry. It has been estimated that about 60 peptide drugs were approved and generated annual sales of around US$ 13 billion (about 1.5% of all drug products) in 2010 alone. The numbers areincreasing significantly with a current continuous annual growth rate between 7.5% and 10%[10]. Peptide-based therapeutics possess a long and secure future as a result of the following merits:

- There is an overall increased acceptance of protein therapeutics by physicians, pharmaceutical corporations and patients. For the past three decades, in the United States alone, the number of peptide new chemical entities (NCEs) entering clinical study per year per decade has increased by about 1300% (from 1.2 per year in the 1970s to 16.8 per year so far in the 2000s)[15]. This increased acceptance has been partly attributed to the development of technological solutions to problems such as short half-life and delivery of the peptide molecules, previously encountered with the application of peptides as drugs[16]. Additionally, the risk of unforeseen side-reactions is unanticipated or at best reduced for food-derived peptide drug candidates. This is because most food proteins and peptides have a long history of use and aregenerally regarded as safe[13]. Also, the existence of a structural relationship between peptide drug candidates and their safe physiologically active parent molecules substantially reduces the risk of unforeseen side-reactions. This is best demonstrated in the fact that there is an over 20% probability of regulatory approval for peptide NCEs, a rate which is double to that of small molecules[12].
- Naturally, peptides play key physiological roles in the body as peptide hormones, chemokines and cytokines. Since peptides are composed of metabolically and allergenically tolerable amino acids, they are generally safe and non-toxic. Usually, any known side-effects with peptide drugs have often been related to dosage or local reactions at the injection site[12].
- Peptide-based therapeutics are effective in addressing a wide array of medical disorders including indications in cancers and tumours, metabolic disorders, cardiovascular health and infectious diseases. Peptides are structurally diverse, have wide spectrum of therapeutic action, low biodeposition in body

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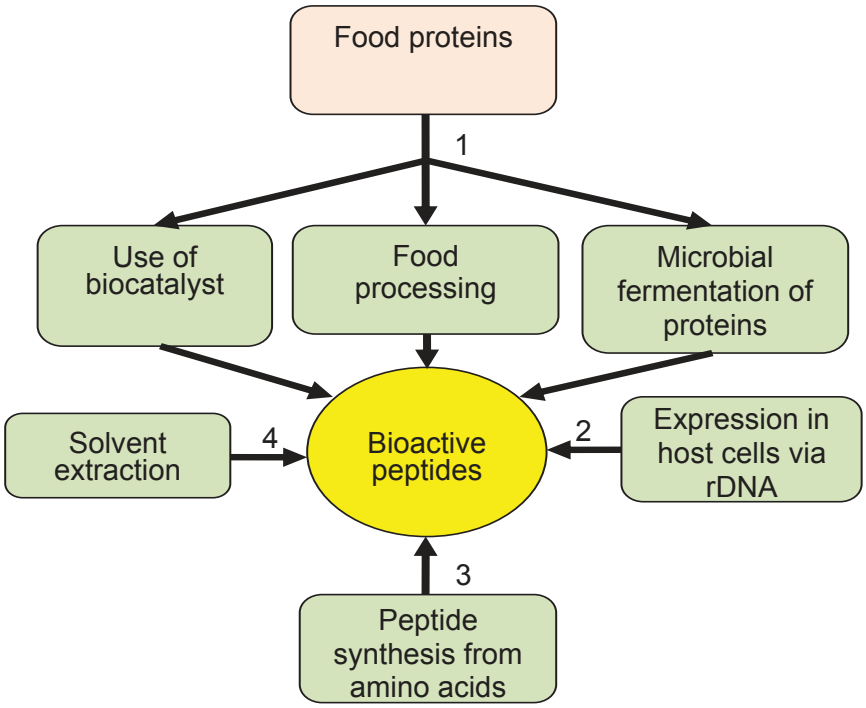

Figure 1: Alternative routes of production of bioactive peptides.

tissues and high biospecificity to targets[13].

Apart from their use as active ingredients, peptides also have the ability to be used as excipients in drug formulations for modification of biological activity, targeted delivery or transport across cellular membranes.

Production and therapeutic applications of bioactive peptides

Broadly, biologically active peptides are obtained via four approaches: 'up-down degradation', 'whole expression', 'bottom-up' construction or 'whole extraction' (respectively, pathways 1–4 of Figure 1). However, for the purpose of this review, a distinction has been made between the terms 'bioactive peptides' and other therapeutic peptides. The term 'bioactive peptides' has been confined to food protein-derived peptides which trigger physiological biological activities in living body systems, to the exclusion of chemically synthesied or whole isolated therapeutic peptides.

Discussion

The authors have referenced some of their own studies in this review.

The protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed.

Therapeutic applications of bioactive peptides

A considerably huge amount of scien-tific research data exists to demonstrate the therapeutic potential of bioactive peptides, some of which are human studies. Some of the physiological effects of peptides are shown in Table 1 and others discussed briefly below.

Antimicrobial peptides

Several peptides have been identified as having antimicrobial properties. Like innate host defence peptides in multicellular organisms, these peptides are relatively short (between 10 and 50 amino acid residues), hydrophobic and cationic[13,17,18]. Natural compounds with antimicrobial activities act in diverse mechanisms; however, most antimicrobial bioactive peptidesact either by penetrating and disrupting microbial membrane integrity or by translocating across the membrane and acting on internal targets[17,18]. Antimicrobial peptides have been identified from many sources, especially from milk protein hydrolysates. Recently, a very potent antimicrobial peptide has been identified and obtained from cleavage of milk whey proteins such as lactoferrin[19]. Lactoferrin has attracted much interest in recent years. It is a potent antimicrobial agent against several enveloped and naked viruses such as rotavirus, enterovirus and adenovirus[20]. Further, tryptichydrolysate fractions of β-lactoglobulin whey proteins have been demonstrated as potent inhibitors of *Listeria monocytogenes* and *Staphylococcus aureus.* Two anionic peptides (Ile-Asp-Ala-Leu-Asn-Glu-Asn-Lys and Thr-Pro-Glu-Val-Asp-Asp-Glu-Ala-Leu-Glu-Lys) exhibited antimicrobial activity against the proliferation of *Listeria* and *Staphylococcus*[21]. In another recent study, a randomized, controlled and blind study was performed on 60 human subjects to investigate whether and how consumption of goat milk cheese affects *Helicobacter pylori* activity in the stomach of these infected persons. The findings indicated that although the consumption of goat and cow milk (as cheese) did not reduce the activity of *H. pylori* in the stomach of infected persons, both cheeses helped improve subjects' overall gastrointestinal well-being[22].

Conclusively, antimicrobial peptides are effective against a wide range of microbial hosts, including Gram-negative and Gram-positive bacteria, fungi, viruses and parasites. As such, not only are theypotential natural biopreservatives but are also effective in combating the rapidly increasing incidence of multidrug-resistant infections

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whilst acting as alternate strategies to replace conventional antibiotics in controlling bacterial infections.

Anticancer peptides

Antitumour and anticancer drugs top the number of drug candidates being developed by pharmaceutical companies since human cancer is one of the most important causes of death in the many developed countries. Although oncology research is well advanced and has enhanced our understanding of cancers, the therapeutic opportunities are still limited owing to the difficulty in selective targeting of cancer cells leaving healthy ones. Effective cancer therapies can utilize biomolecules that target tumour-associated antigens expressed on cancer cells and directly modulate the proliferation and survival of these cancerous cells[23]. Several anticancer peptides have been identified in a range of food protein hydrolysates. For example, recently, a novel anticancer peptide was identified via pepsin hydrolysates of proteins from the shellfish *Mytiluscoruscus*. The hydrolysates were purified and the peptide (with sequence identified as Ala-Phe-Asn-Ile-His-Asn-Arg-Asn-Leu-Leu) effectively induced cell death on prostate, breast and lung cancer cells but not on normal liver cells[24]. Further, antioxidant and anticancer peptides rich in tyrosine, lysine, arginine, phenylalanine and histidine have been obtained via alcalase treatment of solitary tunicate (*Styelaclava*) proteins. Purification of the hydrolysateby gel-filtration gave two peptide fractions with antioxidant and anticancer activities each. The anticancer peptides significantly inhibited human stomach adenocarcinoma cell line (AGS), colorectal adenocarcinoma cell lines (DLD-1) and HeLa cells[25]. With their great potential in killing or controlling the growth and proliferation of cancerous cells, bioactive peptides with anticancer properties have significant health-promoting effects that can be harnessed in the pharmaceutical industry.

Antioxidative peptides

The highly unstable and reactive nature offree radicals and reactive oxygen species (ROS) in the body results in cell damage and consequently leads to diseases such as hypertension, cardiovascular, cancer, diabetes mellitus, and neurodegenerative and inflammatory diseases[26]. Antioxidants protect the body by scavenging free radicals and ROS and also inhibiting lipid peroxidation reactions, thus preventing oxidative damage. Several studies have established the antioxidant properties of some peptides derived from different protein sources[13,14,27–33]. A very potent antioxidant has been identified from hoki frame protein hydrolysates by the use of pepsin, and this peptide (with the sequence identified as Glu-Ser-Thr-Val-Pro-Glu-Arg-Thr-His-Pro-Ala-Cys-Pro-Asp-Phe-Asn) exhibited lipid peroxidation inhibition levels higher than that of α-tocopherol, a well-known antioxidant used as a positive control. The peptide also effectively quenched different sources of free radicals, including 1,1-diphenyl-2-pycryl-hydrazyl, hydroxyl, peroxyl and superoxide radicals; as well as decreased *t*-butyl hydroperoxide-induced cytotoxicity on human embryonic lung fibroblasts, whilst protecting free-radical-induced DNA damage[28]. Antioxidant peptides have also been identified and purified from sweet potato protein hydrolysates prepared from Alcalase. The peptides obtained were rich in hydrophobic amino acids and also contained high amounts of the antioxidant amino acids His, Met, Cys, Tyr and Phe. The peptides had protective effects against DNA damage through scavenging of hydroxyl radicals and Fe^{2+}chelation[34]. Other antioxidant peptides have been obtained via trypsin hydrolysates of Tilapia frame proteins. The antioxidant peptides were identified as Asp-Cys-Gly-Tyr and Asn-Tyr-Asp-Glu-Tyr and had high hydroxyl radical scavenging activities[26]. Reference is made to Chalamaiah *et al.*[35] and citations there in for comprehensive analyses of antioxidant peptides derived from food protein hydrolysates.

Antihypertensive peptides

Antihypertensive peptides are the most widely studied of all the bioactivities induced by food protein hydrolysates[5]. Angiotensin I-converting enzyme (ACE, peptidyldi-peptide hydrolase, EC 3.4.15.1) is a key enzyme in the renin–angiotensin system. This enzyme regulates extracellular fluid volume and arterial vasoconstriction either by converting angiotensin I to the vasoconstrictor angiotensin II or by inactivating the vasodilator bradykinin. Inhibition of ACE therefore results in a decrease in blood pressure, helping to control hypertension. The worldwide statistics of hypertension has shown that in 2000 the estimated total number of adults with hypertension was 972 million and the number of adults with hypertension is predicted to increase by about 60% to a total of 1.56 billion in 2025[36]. Hypertension is therefore a global high-priority public-health challenge requiring urgent attention with prevention, detection, treatment and control. Although several potent synthetic peptides are in use for the clinical treatment of hypertension and heart failure, they are costly and are attended with numerous obnoxious side effects[37]. Antihypertensive bioactive peptides are a suitable alternative since they are relatively cheaper to produce with none or less attendant side effects[13,14]. Most of the antihypertensive peptides are usually short peptides and have a proline residue at the carboxyl terminal end. Proline residues help the peptide to resist enzymatic degradation enroute the gastrointestinal carnal[3]. For example, the well-known antihypertensive peptides are the lactotri peptides isoleucine-proline-proline (Ile-Pro-Pro) and valine-proline-proline (Val-Pro-Pro) which were obtained from sour milk[5,38]. Clinical trial studies have demonstrated that these two tripeptides affect health positively by reducing both systolic and diastolic

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blood pressure in hypertensive patients[39]. Some new and potent antihypertensive peptides have been isolated from many plant food proteins. For example, a valine-and tryptophan-rich peptide with high *in vivo* anti-angiotensin converting enzyme (anti-ACE) activity was identified and purified from alfalfa white protein concentrate hydrolysate[40]. Further, four potent ACE inhibitory peptides (Ile-Tyr, Arg-Ile-Tyr, Val-Trp and Val-Trp-Ile-Ser), have been isolated from subtilisin digest of rapeseed protein. All isolated peptides lowered blood pressure of spontaneously hypertensive rats following oral administration with maximum effect observed between 2 and 4 h[41]. These and the other antihypertensive peptides appear promising for incorporation into functional foods and pharmaceutical lead drugs where they may be useful in the prevention and treatment of hypertension. Antihypertensive peptides help regulate fluid and salt balance in mammals[42].

Cytomodulatory and immunomodulatory peptides

The progression of disease conditions such as HIV and cancer in immunocompromised individuals is very rapid if not controlled properly. A new approach to therapy and robust macromolecules that modulates the immune system is therefore a major research endeavour. Cytomodulatory peptides are suitable biologically active macromolecules for the modulation of body immune cells. They modulate the viability (e.g. proliferation, differentiation and apoptosis) of different cell types, and together with immunomodulatory peptides might help in the control of tumour development[42]. Research has uncovered peptides from Mozzarella cheese waste whey and these peptides showed significant antiproliferative effect on $CaCo_2$ cell lines[43]. In another study, cytomodulatory peptides were shown to inhibit cancer cell growth while stimulating the activity of immunocompetent cells and neonatal intestinal cells[44]. Immunomodulatory peptides, on the other hand, bolster immune cell functions, improve antibody synthesis and cytochrome regulations and also enhance mucosal immunity in the gastrointestinal system[42]. Several peptides with immunomodulatory properties have been isolated from milk proteins where they trigger either specific (lymphocyte activation and proliferation, antibodyproduction, cytokine expression) and/or non-specific (functions of macrophages, granulocytes and natural killer cells) immune responses[5,45–47].

Derma-pharmaceuticals from bioactive peptides

With respect to skin care, proteins and peptides are important due to their role in modulating cell proliferation, cell migration, cell and tissue inflammation, angiogenesis and melanogenesis[48]. A greater majority of products used in the skin care industry are pharmaceutical compounds. As such, bioactive peptides have a great potential for use as active cosmetic and dermo-pharmaceutical ingredients. Currently, many patents exist for dermatological products obtainable from bioactive peptides. For example, peptides of sequence *X*-Thr-Thr-Lys-*Y*, wherein in the particular *X* is lysine and *Y* is serine, have been patented (US patent no. 6620419) as useful in stimulating healing, hydrating or in all skin treatments. They are especially active against the formation of wrinkles and against all the consequences of skin ageing as well as for dry skin[49]. Another disclosed invention (US patent no. 8071555) is a tetrapeptide with the amino acid sequence Pro-Glu-Glu-*X* (where *X* can be either lysine or isoleucine). This tetrapeptide, as an active ingredient in skin care products are effective in controlling inflammatory skin disorders[50].

Multifunctional peptides and peptides with other bioactivities

Interestingly, some bioactive peptides have demonstrated multifunctional roles, triggering two or three bioactivities. Most of the bioactive peptides shown in Table 1 are multifunctional peptides. Lactoferrin is a classic example of a multifunctional peptide exhibiting antibacterial, antifungal, antiviral, antiparasitic and antitumour activities while accelerating immunomodulatory properties[20]. Other examples include peptides obtainable from ovine α_{s2}-casein f(203–208)because it exhibits antimicrobial activity, antihypertensive and antioxidant activities[19]. There are other useful bioactivities of peptides which have not been covered in detail in this report. Among them include peptides that influence nutritional status and dental health, hypocholesterolemic peptides, antithrombotic peptides, antiulcerogenic peptides, opioid peptides andmineral-binding peptides[10,29,42,43].

Challenges to peptide therapeutics and useful '-omic' tools

The feasibility of pharmacological application of bioactive peptides depends on overcoming some challenges, including reduced biostability of peptides, lack of oral bioavailability, potential and a propensity to aggregate into intractable deposits. Moreover, the correct biodistribution of bioactive peptides once delivered is sometimes hampered as a result of proteolytic attack. Despite these challenges, research is well advanced in overcoming most of these challenges. Chemical modification of peptide backbone has been used to increase the stability of peptides in biological fluids. This is achieved via techniques such as amidation, polymer conjugation and the introduction of disulphide bonds[51–54]. Moreover, new delivery strategies employing nanotechnology have been shown not only to protect peptides from enzymatic degradation but also to improve bioactive peptide delivery to target tissues[55,56]. Computer-based tools such as proteomic and computational peptidomic studies are helpful in enhancing peptide therapeutics research. They allow optimisation

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in the production of bioactive peptides, help in conducting peptide searches, and also help enhance the understanding of interaction mechanisms between receptors and bioactive peptides[57]. These help simplify peptide production by utilising computational (*in silico*) skills to predict peptides that can be obtained from a known food protein-before undertaking wet-laboratory synthesis. With this approach, selection of enzymes, proteins and hydrolysates, as well as study the secondary structure and physicochemical properties of peptides obtained can all be done *in silico*. Examples of peptide Database programs are BIOPEP and PepBank whilst PeptideCutter and POPS are proteolysis prediction peptide systems[57]. Integration of proteomic with peptidomic methodologies in the production of bioactive peptide science will enhance the throughput and productivity.

Conclusion

Bioactive peptides are ubiquitous biomolecules widely abundant and easily obtainable from food proteins. There is no limit therefore to the number of peptides that can be obtained from a single food protein. Each of these peptides may present unique structure and biofunctionalities that can be exploited in the pharmaceutical industry. As research continues to uncover technologies and means to overcome challenges to the use of peptide therapeutics, the prospects of food-derived bioactive peptides will likely fuel in the pharmaceutical industry an exodus from small molecules and biologics to bioactive peptides.

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