Bioavailability of Food Bioactive Peptides

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Bioactive peptides have been recognized as an important category of functional food ingredients, which exert a potential impact on human health beyond their nutritional value. However, although peptides have been demonstrated to exert multiple benefits by biochemical assays, cell culture, and animal models, the ability to translate the new findings into practical or commercial uses remains delayed because of several challenges that require to be addressed. Researchers have found that the in vitro bioactivity of peptides do not always correlate with in vivo effects. This is due to the fact that physiological effects of peptides depend largely on their ability to remain intact after digestive process. The peptides that resist the digestion and arrive intact at the intestinal absorption site can have a local function or may be able to cross the epithelium, enter the bloodstream, reach the target organ, and have a systemic effect. Therefore, as much as it is in pharmacology research, investigating the stability and bioavailability of bioactive peptides has become a new trend in the current functional food field.

Keywords: digestion ; absorption ; food peptides ; bioavailability ; biological activity ; encapsulation

1. Introduction

In the last few years, food-derived bioactive peptides have attracted the interest of scientists because of their safety, low cost, and benefits on health beyond the nutritive role. Bioactive peptides have been demonstrated to positively affect the major body systems, notably, the cardiovascular, digestive, endocrine, immune, and nervous systems, while minimizing the risks of chronic disease development [1]. Thus, they have become promising ingredients for functional foods and nutraceuticals [2][3]. A lot of in vitro biochemical assays, cell models, and animal models have been optimized and applied for testing the bioactivity of these food bioactive peptides. However, although the research on the development of peptides-enriched products has notably increased, the ability to translate the new findings into practical or commercial uses remains delayed. Among the major reasons behind this delay, one of the most important is the lack of correlation of the in vitro bioactivities of peptides with in vivo functions due to their low bioavailability following oral administration [4]. Peptides need to resist the action of digestive enzymes during their transit through the gastrointestinal tract and cross the intestinal epithelial barrier to reach intact the target organs where peptides can exert their health-promoting effects [5]. Thus, when studying the effects of bioactive peptides in our organism, it is important to assess their under-digestive conditions, and if the peptide is absorbed, it is necessary to evaluate its distribution, metabolism, and excretion behavior [6][7]. In this review, the most current evidence on the in vitro and in vivo models designed to evaluate the digestibility and bioavailability of food bioactive peptides is summarized, focusing on those limiting factors affecting both peptides resistance to digestive conditions and absorption capacity.

2. Bioavailability of Food Peptides

2.1. Digestibility of Food Peptides

Human digestion is a complex process that involves the concerted action of digestive enzymes on dietary ingredients. In the case of digestion of food proteins, several factors influence this process such as the type of proteins, gastric and intestinal pH, activity of digestive enzymes, endogenous secretions, and motility [8]. Digestion is considered a vital process for life because nutrients released from ingested foods are used by the body as an energy source for cell maintenance and growth [9]. During the digestion of food proteins, peptides and amino acids are liberated, acting as signals of gastric or intestinal motility and pancreatic secretion, and/or exerting local and systemic physiological functions.

Digestion starts with a short food chewing step in the mouth, which is relevant for the complete digestive process, particularly for the gastric emptying rate [10]. The food bolus resulting from mechanical and enzymatic degradations in the mouth is transported through the esophagus to the stomach by peristaltic movements. Once the bolus reaches the proximal part of the stomach, it is mixed with the gastric juice, which is mostly composed of hydrochloric acid (HCl), pepsin and lipases responsible for protein and lipid digestion, respectively, and mucus that protects the mucosal surface.

In the distal part, peristaltic movements allow breaking large food particles into smaller ones by grinding and mixing gastric contents. The stomach ends at the pylorus that pumps small particles (chyme) to the duodenum, while the largest particles are maintained in the stomach for further digestion. Once the chyme enters the duodenum, its acidic pH is neutralized by sodium carbonate ($NaHCO_3$) until reaching a pH appropriate for the activity of pancreatic (proteases, amylases, and lipases) and intestinal enzymes, which are responsible for the subsequent digestion of molecules contained in the chyme. Bile produced by the liver contributes to lipid digestion by emulsifying dietary fats into small droplets that favor the activity of lipase. Once digested, released nutrients are available for their absorption by villus enterocytes through different transport mechanisms, and non-absorbed material travels down to the large intestine. In the colon, water and electrolytes are absorbed, bile salts are reabsorbed, and non-digested polysaccharides and proteins are fermented by colonic microbiota, releasing new degradation products. Finally, at the end of the large intestine, the formation, storage, and elimination of feces occurs [11].

2.2. Absorption of Food Peptides

Absorption of most of the digestion products occurrs in the jejunum, where chyme enters from the stomach, and it is further broken down into nutrients (including peptides, fatty acids, mono- and oligosaccharides, vitamins, and minerals) that cross the intestinal wall, reaching the systemic circulation. Traditionally, it was thought that once ingested, all peptides and proteins were hydrolyzed by digestive enzymes to their constituent amino acids that were absorbed across the intestinal epithelial barrier. It was also believed that proteins and peptides were only absorbed under pathological conditions. However, in the last few years, it has been found that many peptides are absorbed by intestinal cells under normal conditions, being detected in both newborn and adults' bloodstream and/or target organs where they exert their biological activities [12][13][14].

To date, four different routes of peptides absorption have been described: paracellular diffusion, transcellular passive diffusion, transcytosis, and carrier-mediated transport. Following, the main characteristics of these absorption pathways and examples of peptides using them are summarized.

1. a) Paracellular diffusion involves the movement of molecules via water-filled pores/channels between cells. Approximately 0.01–0.1% of the total intestinal surface area consists of water-filled pores that corresponds to 200–2000 cm^2. This surface is large enough for the absorption of small quantities of peptides (pM–nM range); thus, it is adequate to exert their biological activity [15]. The paracellular pathway is regulated by tight junctions (TJs) that separate the apical and basolateral membranes of the epithelial cells. TJs are multiprotein complexes containing zonula occludens-1, occludin, and claudin proteins that form a firm biological barrier restriciting the paracellular flux of water, ions, and solutes [16]. TJs prevent the crossing of substances through the space between plasma membranes of adjacent cells, and they restrict the process of polar macromolecule penetration [17]. Therefore, peptides passing through the intestinal epithelium enter the cells by diffusion or active transport. This transport is mainly dependent on the physicochemical properties of the peptide such as molecular weight and ionic charge [15]. Thus, this route has been reported as that preferred by hydrophilic negative charged low-molecular-weight peptides [18][19][20]. A variety of bioactive food oligopeptides have been found to be transported by passive diffusion via paracellular TJs (see the review of Xu et al., [21]). Moreover, this route was used by the 43-amino-acids peptide lunasin and its fragment RKQLQGVN, which is released during lunasin's gastrointestinal digestion, to cross intestinal epithelial barrier [22].
2. b) Transcellular passive diffusion involves the transport of molecules through apical and basolateral membranes in a concentration-based and energy-independent manner [23]. The transport of bioactive peptides through passive diffusion is dependent on peptide characteristics such as size, charge, and hydrophobicity [24] Thus, because of the composition of the cell membrane by a lipid bilayer, it is widely accepted that lipophilicity plays a key role in this transport mechanism. While hydrophilic peptides prefer paracellular diffusion to cross the intestinal epithelium, transcellular transport is the chosen route by lipophilic peptides. Other factors, such as the peptidic chain length and number of polar groups also seem to determine the passive diffusion of bioactive peptides. Moreover, the transcellular absorption of a peptide depends on the energy required to break water–peptide hydrogen bonds, allowing the molecules to enter the cell membrane [25].
3. c) Transcytosis involves the energy-dependent transport of material from one side of the polarized cell to the other. This route includes apical endocytotic uptake, transcytotic transport via internalized vesicles called endosomes, and basolateral secretion [26][27]. Since peptides need to interact with the apical lipid bilayer of epithelial cells through hydrophobic interactions before being internalized by the cells, transcytosis seems to favor the transport of long-chain (more than four amino acid residues) and hydrophobic peptides [28][29]. Thus, a recent study has suggested that the high content of hydrophobic amino acids in the antioxidative peptide YWDHNNPQIR could determine its transport across Caco-2 cell monolayers via transcytosis [30]. In addition to the importance of hydrophobicity, other factors have also been recognized as determinants in the transcytosis transport of peptides. Thus, cell models have reported that

the number of polar groups and the net charge of peptides, especially the positive charge, show positive effects on their transcytosis transport [31][32].

4. e) Carrier-mediated transport involves the movement of peptides against the concentration gradient, which is mediated by specific cell membrane proteins that function via anti-, sym-, and uniporter mechanisms. Antiporters translocate peptides in opposite directions, whereas symporters transport them via cotransport in the same direction over the blood membrane. Uniporters function unidirectionally, without cotransport . This transport system is dependent of susbtance concentration, susceptible to inhibition, and specific to the molecules' structure [33]. Among peptide carriers, transporter 1 (PepT1) is a high-capacity and low-affinity carrier that drives peptides from the gastrointestinal lumen into the intestinal epithelium in a proton gradient and membrane potential-dependent manner [34][35][36][37]. Although it has been described that PepT1 preferentially binds short-chain bioactive peptides, specially di- and tri-peptides, with neutral charge, and high hydrophobicity, it has also been found to be able to recognize dipeptides with an extreme bulk or two positive charges [38]. However, PepT1 is unlikely to bind hydrophilic or hydrogen regions [39]. Recently, Wang and Li used a Caco-2 cell monolayer model to study the transport routes chosen by casein-derived peptides [40]. These authors found that PepT1 was responsible for transporting low molecular weight peptides, while high molecular weight casein peptides crossed the intestinal barrier through paracellular diffusion. Moreover, the bioavailability of peptides transported by PepT1 was higher than those transported through the paracellular route. PepT1 has also been described as the carrier of angiotensin converting enzyme (ACE) inhibitory peptides IPP and LKP, which were released from milk β-casein and fish/chicken muscle protein, respectively [41], as well as of other food bioactive peptides [101,112–116]. In addition to PepT1, other peptide carriers present in the basolateral membrane have been suggested to participate in the transport of hydrolysis-resistant small peptides into blood. However, this fact has not been proven yet [117,118].

2.3. Effects of Gastrointestinal Endogenous Protein-Derived Peptides

Gut endogenous proteins represent a larger and more constant supply of protein in the gastrointestinal tract in comparison with dietary protein [42]. They are constituted by gastrointestinal tract epithelial turnover, gut microflora proteins, and soluble secreted proteins such as mucins, digestive enzymes, hormones, serum albumin, immunoglobulins, and lysozymes, among others [43]. Although these endogenous proteins have been exhaustively studied to estimate the dietary amino acid requirements and digestibility, the data on their potential as a source of bioactive peptides are still scarce. However, given the high amount of endogenous proteins present in the gastrointestinal tract, a wide array of potentially bioactive peptides are expected to be liberated during the digestion process. In a preliminary in silico gastrointestinal digestion prediction model, 26 gut endogenous proteins were evaluated as a source of bioactive peptides [44]. The total number of bioactive peptides predicted to be released ranged from 1 (secretin) to 39 (mucin-5AC), of which ACE-inhibitory peptides were the most frequently observed. These results were confirmed by an in vitro digestion assay of endogenous proteins, resulting in the release of a high number of antioxidant, ACE, and DPP-IV inhibitory peptides [45][46]. Similarly to dietary bioactive peptides that have been demonstrated to bind to specific receptors in the gut modulating gut motility, satiety, and the secretion of gastrointestinal endogenous proteins, peptides released from these endogenous proteins might also have effects on gut physiology and functions [47]. Therefore, little modifications of both dietary and endogenous sources of bioactive peptides offers a great opportunity to modulate gut processes.

2.4. Strategies to Improve Bioavailability of Food Peptides

Once the mechanisms involved in the transport of bioactive peptides and the factors influencing their absorption are known and understood, it is possible to design valuable strategies that improve the bioavailability of peptides and maintain their potent in vivo bioactivities. These strategies aim at achieving the following objectives: (i) reduction of the detrimental effects of food processing on peptides bioactivities; (ii) promotion of the desiderable interactions between peptides and other food matrix components, reducing the undesiderable ones; (iii) protection of bioactive peptides from gastrointestinal conditions and digestive enzyme activity; (iv) control of the sustained peptides' release directed at their target organs; and (v) improvement of the transport of bioactive peptides across the intestinal epithelium and target cells [188].

3. Conclusions

It has been recognized worldwide that food-derived bioactive peptides are valuable ingredients of functional foods and/or nutraceuticals to promote health and reduce the risk of chronic diseases. However, although oral administration is the preferred route for bioactive peptides, the translation of in vitro activity to in vivo effects when peptides are orally ingested is not always realistic. These discrepancies are due to the molecular characteristics of peptides as well as to both dietary and non-dietary factors. Molecular mass, amino acid sequence, and additional structure modifications are determinant properties for the resistance of peptides to digestive enzymes and the preferred transport route to cross the intestinal

barrier. Among dietary factors, the interactions between peptides and other compounds of the food matrix are considered relevant, since these components may reversibly or irreversibly react with bioactive peptides, modulating their digestibility and/or altering the routes of absorption of peptides, influencing their bioavailability. However, to date, the existing evidence on the effects of the food matrix is still limited. In addition, the behavior of peptides during their transit through the gastrointestinal tract depends on health and pathological conditions that can alter the digestive and absorptive gut environment. Thus, for a better understanding of the in vivo physiological effects of food bioactive peptides, extensive research studies on their gastrointestinal stability and transport are needed. Combined in vitro studies simulating gastrointestinal digestion conditions and cell culture mimicking the intestinal absorptive environment are being optimized, becoming an interesting and valuable approach to confirm the beneficial role of peptides on health at doses that are physiologically relevant. Due to the low bioavailability of most food peptides, efforts are being focused on the design of new strategies that increase their resistance to the action of digestive enzymes during their transit through the gastrointestinal tract and allow the controlled release of intact and active peptides in the target organs where they exert their biological activity. Among these strategies, delivery systems with natural, safe, and biocompatible materials are becoming the most promising; thus, further research should be needed to optimize the encapsulation conditions enhancing the digestibility and bioavailability of food bioactive peptides.

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