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Food peptides: purification, identification and role in the metabolism

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The search for new active molecules from food protein hydrolysates represents a real challenge for animal feed, aquaculture, as fertilizer, but also in cosmetics and pharmacological fields. The constant discovery of molecules with biological activities continually brings new insights into the complex mechanisms involved *in vivo*. However, it remains difficult to conclude about the structure, amino acid composition, size or the physicochemical properties necessary for the activity. This review will highlight the research carried out to determine the physiological role of these peptides and the development of new tools for their production and study.

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Introduction

The multiplicity of peptide sequences generated by the hydrolysis process provides a wide range of structural and physicochemical properties that can help, using technological processes such as ultrafiltration membranes, to separate them by family properties and to draw the necessary conditions to maintain the activity [1]. There are yet no general rules for determining the best hydrolysate with the most important activity. Each raw material, each enzyme and each hydrolysis conditions will give different final peptides. The relationship between the sequence and activity is still studied and represents a basic research for the discovery of the complex mechanism of action of these biological activities [2–5]. A set of recent reviews described the high biodiversity of bioactive peptides from animal and plant proteins [6,7•,8••,9•,10]. In this short review, we will focus on the recent evolution of several research fields related to protein hydrolysis such as the role of bioactive peptides in energy homeostasis, the importance of peptidomics and the development of controlled bioprocesses for the separation of bioactive peptides.

Dietary proteins as sources of bioactive peptides involved in energy homeostasis

In economically developed countries, eating behaviors, characterized by an increase in energy intake which causes an increase in body mass, led to the metabolic syndrome. This syndrome predisposes to many diseases such as type II diabetes, a deregulation of food intake, hypertension, chronic inflammatory bowel disease (IBD) [11] and became one of the most alarming public health problems of this century. Digestion has for a long time been considered as a black box producing unit nutrient compounds such as amino acids involved in metabolism and indispensable to the physiological function of the body. In recent years, an abundance of studies have shown the involvement of peptides generated during the digestion in many physiological functions [12] and especially in energy homeostasis and consequently in the 'metabolic syndrome' and in its various symptoms.

Regulation of food intake

During a meal, the presence of nutrients in the gastrointestinal tract results in the production of signals that inform the brain about their qualities and quantities. Among these signals, peptide hormones secreted by enteroendocrine cells such as cholecystokinins (CCK) and glucagon-like peptide 1 (GLP-1) were particularly studied and are of great interest for the development of new drugs and dietary supplements targeting obesity [13]. During digestion, the products of hydrolysis of food proteins are known to have a high satiating power, greater than that exercised by carbohydrates or lipids, stimulating the secretion of gastrointestinal hormones [13]. Thus, recent work has identified peptides able to exert a strong stimulation on the secretion of hormones involved in the regulation of food intake [14]. Another promising

approach in the research for natural molecules that can be used in functional foods concerns the identification of peptides exerting agonistic action to that of CCK and GLP-1 by binding to their cellular receptors distributed in the gastrointestinal tract and central nervous system. Thus, studies have identified peptides able to bind to these receptors and to exert *in vitro* and *in vivo* agonistic action [15–17]. In this way, Pupovac and Anderson [18] evidenced in rats that peptides arising from digestion contribute to satiety by independent activation of both opioid and CCK-1 receptors.

Glucose metabolism

GLP-1, an intestinal incretin, well known to be involved in the food intake regulation, also acts on the functionality of pancreatic islets mainly by stimulating insulin secretion [19] and slowing the glucagon secretion. An interesting approach is to assume that peptides able to cross the intestinal barrier will be also able to inhibit the action of the dipeptidyl peptidase IV (DPP-IV), a multifunctional enzyme mainly involved in the degradation of GLP -1 and thus consequently in the regulation of glucose metabolism. The incretin effect is drastically reduced or lost in type 2 diabetes mellitus (T2DM). Lately, DPP-IV inhibitors have indeed been considered as innovative molecules for the treatment of T2DM enhancing the GLP-1 activity and recovering the incretin effect. Therefore, incretin-based therapy using DPP-IV drug inhibitors (gliptins) is one of the most recent alternative treatments of T2DM as lately reviewed [20]. Nowadays, several works focus on the strategy to identify 'natural' peptide inhibitors of DPP-IV activity as an alternative to synthetic drugs. Such peptides could be obtained by processing food (protein hydrolysis and microbial fermentation) or by proteolysis occurring during digestion. Numerous studies had first reported that milk derived products induced DPP-IV inhibition *in vitro* [21,22]. Other studies evidenced DPP-IV inhibitory peptides from different protein sources like rice bran hydrolysates [23], fishes or macro-algae [24,25]. A recent study demonstrated, *in vitro*, for the first time that a cuttlefish hydrolysate submitted to gastrointestinal digestion could extend the incretin effect *via* two mechanisms: the stimulation of intestinal GLP-1 and the inhibition of intestinal DPP-IV activity [26•]. These results could be linked to those obtained with a porcine skin gelatin hydrolysate, harboring a DPP-IV inhibition activity *in vitro*, and improved glucose tolerance in diabetic rats in correlation with the increase of insulin and the GLP-1 plasma levels, the inhibition of plasma DPP-IV activity and the decrease of the plasma glucagon level [27]. Recently, new prospects demonstrated that the computer-driven screening of bioactive peptides and the *in silico*/*in vitro* combinatory approaches are attractive tools for the more rapid discovery of new bioactive peptides [28,29].

The need to identify peptidomes to understand peptide bioactivities

Long time challenging, the identification of all peptides of a complex mixture has become easier, rapid and accurate with the emergence of 'omics' techniques [30••]. In the field of bioactive peptides, peptidomics is by definition the analytical method of choice for the structural elucidation of peptides and of their post-translational modifications. Peptidomics is defined as the comprehensive characterization of peptides in a sample [31•]. Practically, peptidomics is a several steps procedure. First, pre-purification methods are used to separate peptides from all other molecules (carbohydrates, proteins, lipids, salts…). Then a combination of peptide fractionation techniques leads to the separation of the different peptides based on different physico-chemical properties (size-exclusion chromatography (SEC), liquid chromatography (LC), capillary electrophoresis (CE), gel-free isoelectrofocusing (GF-IEF)). Finally, Tandem mass spectrometry (matrix assisted laser desorption/ionization-tandem mass spectrometry (MALDI-MS/MS), electrospray-mass spectrometry (ESI-MS)) and bioinformatics are used to identify and quantify all peptides in the complex mixture [32,33,34••,35,36]. Today, the identification of peptides relies on the MS-based fragmentation methods (collision induced dissociation (CID), electron collision or transfer dissociation (ECD and ETD, respectively)), the data acquisition speed and the accuracy of the most recent mass spectrometers [37•]. Moreover, the MS-data acquire way (data dependent acquisition (DDA) or data independent acquisition (DIA); positive or negative mode; ion-mobility), the repetition of biological and technical experiments, the bioinformatics tools used to match the experimental MS-data and the protein databases used have a direct impact on the exhaustive characterization of peptides [38]. Although the MS-based quantification methods (label-free, isobaric and isotope labeling) are available and validated [34••], the relative quantification of bioactive peptides remains rare and therefore the batch-to-batch reproducibility is too less often reported [39]. Software (owned MS-manufacturer, software developed by academic laboratories, free-of-charge or costly) of identification and quantification of peptides are numerous, and all having advantages and drawbacks that invite the user to integrate the bioinformatics results in a metascoring system to obtain a comprehensive view of the peptide heterogeneity of a sample. Even if peptidomics is well adapted some limitations exist such as ranging from the small peptides (<5 amino acids) that are challenging to identifying by tandem-MS due to the low number of MS fragments obtained [40]. The hydrophobic and hydrophilic nature of peptides also complexifies their separation and requires the use of several methods of peptide fractionation. To end with, databases and bioinformatics tools need to be upgraded to limit the false positive rate that is 100 times higher in peptidomics than for proteomics [41].