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Review

Protein digestomics: Integrated platforms to study food-protein digestion and derived functional and active peptides

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ABSTRACT

In the global perspective of "foodomics", tracking the fate of food proteins upon gastrointestinal (GI) digestion assumes a particular relevance, because the products of protein degradation comprise possible functional components with positive (health-promoting activity) or adverse (allergy, intolerance, toxicity) effects on human health. Here we review the recent contributions of the 'omic' sciences to characterize the protein "digestomes" and, in perspective, to validate the experimental models of digestion, with the ultimate scope of elucidating the kinetics and dynamics of dietary proteins.

Contents

1. Introduction

1.1. The metabolic fate of dietary proteins

Exactly a century ago, based on a series of interrelated investigations, Van Slyke and Meyer formulated several hypotheses about the metabolic fate of dietary proteins [1]. Pioneering an entire stream of future studies, the researchers emphasized that intact proteins, even as large as egg ovalbumin, can be adsorbed in such a large amount to be detected unhydrolyzed in the urine. Further investigation showed that the entrance of intact ovalbumin into the blood circulation is abnormal. Indeed, proteins are extensively hydrolyzed along the stages of gastrointestinal (GI) digestion up to free amino acids (AAs) or short oligopeptides.

Between the 1960s and the 1980s [2] and in the past decade, significant advances have been made in the physiology of (oligo)peptide digestion and absorption. As a result, the number

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of investigations focused on food-protein digestion is steadily increasing (Fig. 1). Currently, because of the progress in food science, food structure is much more understood than some years ago and methodologies of investigation are more refined.

According to a huge amount of recent literature, peptides that are released from food proteins in the gastrointestinal (GI) tract can escape digestion and can be absorbed into the intestinal lymphatic system. Exogenous dietary peptides are presumed to exert potent physiological activities, for example, by interacting agonistically or antagonistically with the same targets as their endogenous counterparts, which they often share common structural traits with [3]. Even though food-derived peptides can elicit hormone-like functions locally in the GI tract, in general, bioactive molecules are required to flow into the blood to carry out some specific activities at a systemic level. Despite the relevance of the matter and the extensive research efforts, the issues related to the resistance, absorption/distribution and possible biological activities of food-derived peptides remain far from being elucidated and are fervently debated. In other words, those patterns that in drug metabolism are referred to as "pharmacokinetics" and "pharmacodynamics" are still elusive for dietary peptides.

Even recently, Foltz et al. [4] questioned the possible role of biologically-active peptides and the common techniques aimed at their detection in blood. The great majority of the studies about food-derived bioactive sequences largely neglects the low bioavailability of peptides that results from their poor biokinetics properties. Peptides are susceptible to extensive hydrolysis by sequential gastric, pancreatic and small intestinal brush-border-membrane (BBM) peptidases. The sequences that are taken up at nano-molar or pico-molar concentrations can undergo fast hydrolysis in blood. As an example, lactokinin, a peptide arising from β-lactoglobulin, β-LG f(142–148) that exhibits angiotensin converting enzyme (ACE) inhibitory activity *in vitro*, was demonstrated to be insufficiently stable against GI proteases [5]. Diminished by simulated digestion, the low residual anti-ACE activity was completely repressed when the peptide was incubated with human blood, proving that serum proteinases and peptidases further degrade circulating peptides [5].

However, a body of evidence proves that dietary peptides that survive luminal digestion and endure enterocyte BBM hydrolysis can be detected in measurable amounts in the peripheral blood and urine [6]. For example, κ-caseinglycopeptide and N-terminal of α_{s1}-casein were detected by ELISA and microsequencing in the blood of human volunteers after test feeding with milk and yogurt [7].

Food-derived peptides have also been quantified in biological fluids. For example, the blood levels of several ACE-inhibitor peptides were quantified in volunteers, who had orally taken up these peptides, using LC-ESI-QqQ-MS/MS [8]. However, it has to be underlined that only a relatively small number of studies demonstrate the occurrence of dietary peptides longer than 5–6 AAs in blood.

IgE-mediated food allergies probably represent one of the clearest clues that large polypeptides can survive GI digestion to be then delivered to the serosal side of the gut epithelial barrier. Quite sound evidence correlates digestion stability and allergenic potential of the majority of food proteins [9,10]. Although sentry-like dendritic cells may, in principle, trigger sensitization at the level of the intestinal lumen [11], it has been demonstrated definitively that immunologically-active peptides cross the epithelium *via*

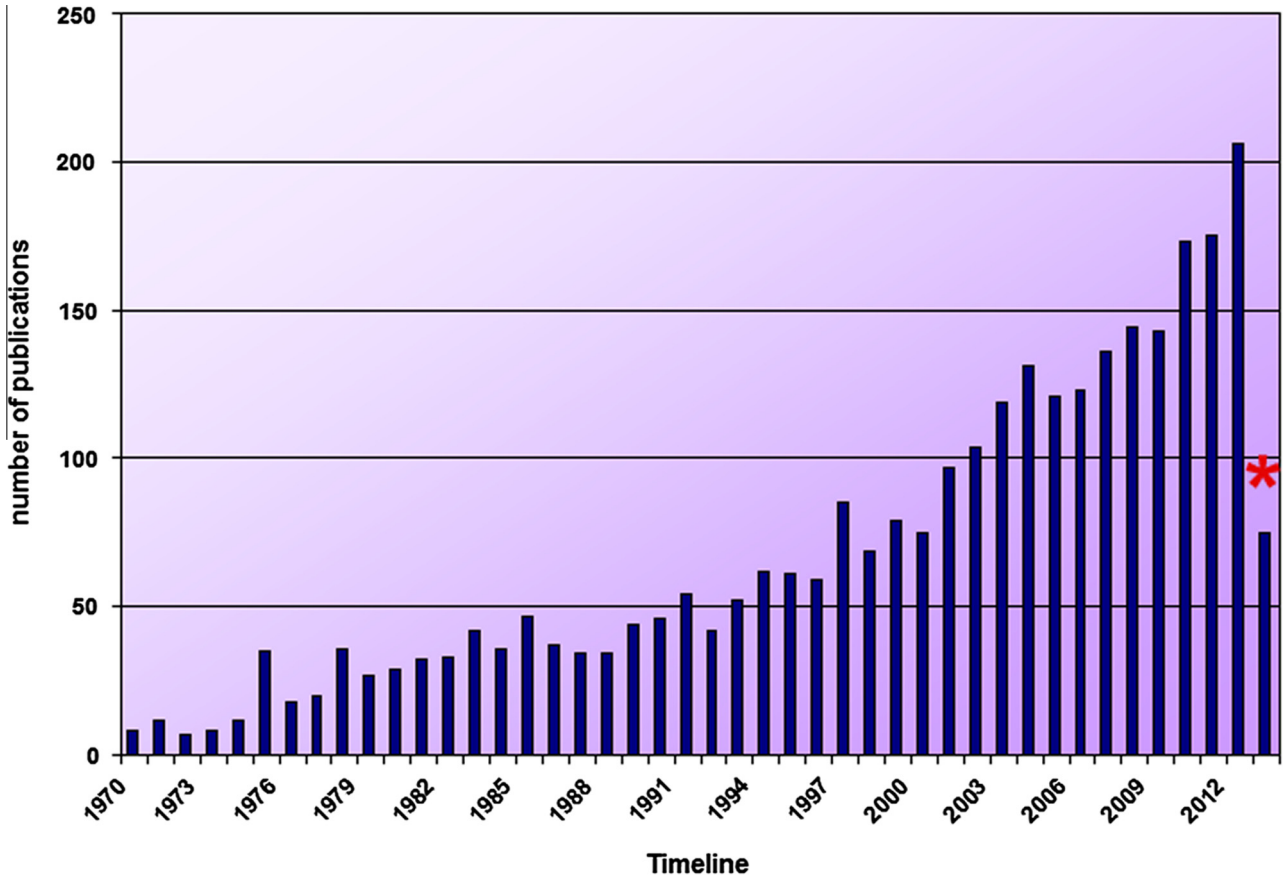

Fig. 1. Number of scientific papers retrieved in the PubMed database using the search key "food protein digestion" within the period 1970–2013. The steadily increasing trend shows the growing interest of researchers in this topic. *The datum concerning 2013 is limited to the first four months.