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Review

Use of brush border membrane vesicles to simulate the human intestinal digestion

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ABSTRACT

The intestine presides over a series of vital functions in the human body, among which the digestion/absorption of nutrients. Despite their major digestion role, the impact of the enzymes of the luminal intestinal surface on food components has been considered in relatively few experiments of simulated gastrointestinal digestion. In contrast, the identification of proteolitically stable peptides which survived digestion in multiphasal models that also included a step with small intestinal brush border membrane (BBM) peptidases has provided physiologically consistent results. Herein, we critically review the use of BBM enzymes to simulate the intestinal digestion of dietary polypeptides. Addressing the controversial issue of the in vitro–in vivo correspondence of the digestion models, the review emphasizes the need to establish consensus protocols to simulate the intestinal step, for instance using the BBM hydrolases at least in a selected number of cases. The factors that have limited the development of relevant models of intestinal degradation are discussed together with hints to possible alternatives, forthcoming approaches and future perspectives to reproduce the physiopathology of the human small intestine.

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1. Introduction

Due to a series of constraints in conducting human trials, several models of simulated gastrointestinal digestion have been deviced to assess the digestibility of dietary macromolecules as well as the bioaccessibility and bioavailability of nutrients. The sequential gastric and duodenal decomposition of foods has been reproduced in a number of different ways, in many cases even using physiologically non-relevant parameters. Therefore, the comparison of the results as well as the standardization and the validation of the procedures remain main concerns (Kopf-Bolanz et al., 2012; Hollebeeck, Borlon, Schneider, Larondelle, & Rogez, 2013; Minekus, Alminger, Alvito, Ballance, et al., 2014). Despite the major digestive role accomplished by intestinal epithelium, most of the multi-step models of human digestion completely omitted the corresponding phase of food degradation. For instance, the in vitro static digestion model recently harmonized does not include yet a simulated intestinal (jejunal) compartment, although the authors were explicitly aware that an additional step with hydrolases from intestinal mucosa would be required to mirror human physiology (Minekus et al., 2014). Intestinal phase of digestion is very difficult to be modeled. One of the

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approaches could be the use of isolated small intestine brush border membrane (BBM) vesicles. Starting from a set of soundly established physiological aspects and examining the more or less recent literature on the topic, this review intends to answer the question whether and in which cases the reasonable use of BBM enzymes could be a realistic or, on the contrary, a too simplicistic method to simulate the jejunal digestion of dietary proteins. In particular, we critically discuss selected "successful" studies whose outcomes have been validated by in vivo evidences, as well as shortcomings and actual or possible alternative to the use of BBM enzymes.

1.1. The intestinal mucosa as a "smart" and active border

Food digestion is a multi-scale process. Structured foods undergo macroscopic shearing and mincing in the mouth, besides a preliminary demolition of the starchy matrix. The breakdown of nutrients proceeds at a (macro)molecular level in the stomach and in the duodenum where, among the other food components, dietary proteins are cleaved down into medium- and small-sized peptides. Microscopic intestinal flow and mixing are propaedeutic to the final processing of nutrients, which includes the hydrolysis of oligosaccharides and large lipids as well as the trimming of peptides up to di−/tri-peptides and free amino acids.

Finger-like protrusions, known as villi, carpet the internal surface of the small intestine. Villi are lined by a monolayer of epithelial cells, more than 80% of which is made of enterocytes. Enterocytes are tightly joined polarized columnar cells originating from multipotent stem cells which reside in the intestinal crypts. The apical surface of the enterocytes is constituted by a dense array of microvilli microscopically observable as the brush border. The terms brush border and microvilli are often used interchangeably to indicate the small intestinal surface.

Villi and microvilli increase the intestinal surface area deputed to digestion and absorption of nutrients. Depending on the species, microvilli range between 0.5 and 1.5 μm in length and around 0.1 μm in width and their estimated density varies between 3000 and 7000 units per cell (De Sesso & Jacobson, 2001). In this way, brush border increases the apical surface area by 14- to 40-fold and one squared millimeter of intestinal mucosa can contain up to 200 million microvilli (Quaroni & Calnek, 1999). Combining the length of the intestinal segments with the folding of *plicae circulares* and with the microstructural arrangement of the epithelium, the gut of an adult man has a total surface area that is comparable to that of a tennis court. According to a recent evaluation the luminal surface area of the human gut could have been overestimated, although remaining strikingly large (Helander & Fändriks, 2014).

As a large interface between the environment and inner body, the intestinal mucosa is constantly exposed to the injury of potential harmful chemical and biological agents. However, several defense systems contribute to limit the massive invasion by loads of luminal antigens.

Nutrients must penetrate a mucus gel coat composed by two layers, one loosely and one firmly adherent, before reaching the epithelium where they can be rejected, transformed or absorbed. The mucus layer is thinner at the level of the distal duodenum and, in particular, in the jejunum if compared to other districts (Atuma, Strugala, Allen, & Holm, 2001). Fuzzy mucin-type glycoprotein filaments radiating from the apical domain of the enterocytes constitute the brush border glycocalyx that gives evidence of a cell hyperpolarization (Maury, Nicoletti, Guzzo-Chambraud, & Maroux, 1995). The glycocalyx also englobes peptidases and disaccharidases synthesized by enterocytes which actively contribute to the final step of food digestion.

The human gut contains the largest mass of lymphoid tissue in the body (gut associated lymphoid tissue, GALT) and produces the highest quantity of antibodies compared to other organs. Overall, these anatomic traits point out that intestinal epithelium is much more than a passive boundary because it accomplishes key functions within the last stage of the digestion process, in addition to act as a "smart" sift to separate the "good grains" (nutrients) from the "chaff" (potentially harmful antigens and pathogen microorganisms).

1.2. The digestive/adsorptive function of microvilli — a brief historical survey

The discovery of the digestive/adsorptive function of microvilli has a rather controversial but extremely fascinating history. In the past, the investigations were carried out mainly by tracking the degradation of dietary proteins along the gastrointestinal tract and monitoring the efflux into portal blood. Although intact egg ovalbumin of dietary origin had been found in blood at a detectable amount, it was early recognized that this was not a physiological event (Van Slyke & Meyer, 1913). According to the "hypothesis of resynthesis" formulated by Otto Funke and supported by eminent physiologists up to the end of the 19th century, peptones arising from gastric and duodenal breakdown of proteins were immediately resynthesized into albumins at the level of the intestinal mucosa. The framework of the resynthesis theory substantially relied on the failure to detect peptones in portal blood soon after digestion. In 1906 Otto Cohnheim attempted to confirm the protein resynthesis, but he obtained the opposite evidence: the intestinal mucosa heavily degraded peptones even into free amino acids. He named "erepsin" (from the Greek verb ερειπειν that means "to break to pieces") what he believed to be the single enzyme responsible of the intestinal hydrolysis (Matthews, 1978). Later investigators found out that the activity of erepsin was quite specific as it hydrolyzed almost exclusively peptones, while it practically had no effect on large proteins. Thus, combined with the evidences obtained a few years before by Künhe that pepsin and trypsin have substantially different hydrolytic actions, it appeared clear that the ingested proteins undergo a compartmental-specific sequence of hydrolytic cleavages that are physiologically finalized to their extensive degradation. With the "polyfistula method" that extended the experiments carried out by Ivan Pavlov with fistulized dogs, Efrim Semenovich London demonstrated that peptides undergo a progressive "erosion" along the small intestine, before being absorbed essentially as free amino acids (Davenport, 1992; Underhill, 1915). The digestive role of the intestinal mucosa proposed by Cohnheim was quickly accepted by the scientific community (Folin & Denis, 1912), to be absurdly disregarded a few years later, substituted by the hypothesis of an intralumen hydrolysis of peptides. However, in the 30s and 40s of the last century, Max Bergmann isolated several peptidases from mucosal "erepsin", characterizing their activity. Bergmann died a few months after the publication of one among his classic papers about the characterization of the activity of peptidases from intestinal mucosa (Smith & Bergmann, 1944), but the discovery of the key digestive role of small intestine had been primed. A few years later Miller and Crane isolated the epithelial BBM preserving the distinctive sub-cellular morphology, thereby localizing the BBM as the site of the terminal digestion of sugars and peptides (Miller & Crane, 1961a; Miller & Crane, 1961b).

In the second half of the 20th century, the microstructural organization of the small intestinal mucosa has been the subject of an intense research that provided a large bulk of information, also including the identification of more than twenty different BBM-associated hydrolases and as many proteins with binding and/or transport functions. The research of those years concerning the protein organization and topology of BBM has been brilliantly reviewed (Holmes & Lobley, 1989).

1.3. The peptidases of intestinal brush border membrane

In 2007, Donowitz et al. displayed a detailed picture of the mouse jejunal BBM combining shotgun proteomic analysis and immunofluorescence (Donowitz et al., 2007). The proteome comprised 570 proteins, among which almost 40 degradative enzymes. Typically, the hydrolases and transport enzymes are organized in membrane-associated protein