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Review

Ribulose-1,5-bisphosphate carboxylase as a sustainable and promising plant source of bioactive peptides for food applications

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ABSTRACT

Background: Plant proteins are well-known precursors of bioactive peptides. In translating the peptides into functional foods, the protein sources need to be sustainable and readily available. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the major enzyme in photosynthesis and photorespiration in plants and some other organisms, and is known to be the most abundant protein on earth. Therefore, RuBisCO is an attractive and sustainable source of bioactive peptides.
Scope and approach: This review discusses the structure, function, composition and technology for plant RuBisCO extraction, as well as the fractionation and known bioactivities of its enzymatic hydrolysate and peptides. Feasibility of industrial scale up and practical application of the RuBisCO peptides in food were also considered.
Key findings and conclusions: Several processes are available for extraction of the RuBisCO subunits and some are simple, fast and adaptable for industrial scale production. Work is however needed on recovery of high protein yields with high purity. Most studies reported that peptides, mostly from the large subunit, from enzymatic hydrolysis of spinach or alfalfa RuBisCO possess antihypertensive, opioid-like, secretagogue and food intake stimulating, antioxidant and antimicrobial activities. These properties demonstrate that RuBisCO can be utilized as a sustainable source of peptides with multiple bioactivities for formulation of functional foods.

1. Introduction

The quest for natural and health-promoting compounds has led to increased exploration of food materials as sources of functional ingredients. Several studies in recent years have highlighted the health sustaining properties of food-derived prebiotic oligosaccharides, bioactive peptides, lipids and phytochemicals (Ale, Mikkelsen, & Meyer, 2011; Maestri, Marmiroli, & Marmiroli, 2016; McClements, Decker, Park & Weiss, 2009; Rizzello et al., 2016; Udenigwe & Aluko, 2012). Notably, bioactive proteins and peptides are emerging at the forefront of functional food trends and potentially marketable bioactive products. In general, sales of peptide-based drugs were valued at about $20 billion and account for about 2% in annual sales of the global drug market (Sun, 2013). Such peptides are typically produced by bottom-up rational design and chemical synthesis or through heterologous expression and secretion in microbial systems. On the other hand, a top-down approach often applied in food research involves using proteins of plants, marine or animal origins as precursors of bioactive peptides (Ejike et al., 2017; Korhonen & Pihlanto, 2003, 2006; Korhonen, Pihlanto-Leppäla, Rantamäki, & Tuomo, 1998; Maestri et al., 2016). Based on this approach, proteins from milk, fish, meat and egg are commonly used as animal sources of bioactive peptides, whereas cereals, oilseeds, legumes and vegetable proteins are the popular plant-based sources. Notably, these sources of bioactive peptides are also primary dietary protein sources in different populations of the world.

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Bioactive peptides are inactive within their parent protein structures until they are released by the action of endogenous or exogenous proteolytic enzymes, or by microbial fermentation (Maestri et al., 2016; Rizzello et al., 2016; Udenigwe & Aluko, 2012). The peptides possess disease preventive and health management properties that include antihypertensive (Martínez-Maqueda, Miralles, Recio, & Hernández-Ledesma, 2012) antidiabetic (Nongonierma & FitzGerald, 2016), antioxidative (Samaranayaka & Li-Chan, 2011), anticancer (Rajendran, Ejike, Gong, Hannah, & Udenigwe, 2017), opioid-like (Teschemacher, 2003), anti-inflammatory (Chakrabarti, Jahandideh, & Wu, 2014), hypolipidemic (Howard & Udenigwe, 2013) and antimicrobial activities (Haque & Chand, 2008). There has been a slow progress in the translation of laboratory results on bioactive peptides into commercial functional food products. Industrial-scale production of the peptides is expected to increase especially with the availability of new and emerging processing technologies. Despite the progress, some factors are perceived as impediments to the translation of bioactive peptides into functional foods. These limitations include bitterness of some peptides, food matrix-peptide interactions, low peptide bioavailability, limited human studies, lack of a consensus on their molecular mechanisms, low peptide yields, and sustainability of their protein sources (Li-Chan, 2015; Udenigwe, 2014). Sustainability becomes very important since the use of food proteins for bioactive peptides production can lead to competition with human food resources with negative impact on food security, especially in developing countries. This paper addresses the latter by discussing efforts on the exploration of widely available natural proteins as precursors for bioactive peptides.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is a bifunctional multimeric plant metabolic enzyme that participates in carbon fixation in the Calvin cycle and photorespiration, depending on its affinity for carbon dioxide or molecular oxygen (Barbeau & Kinsella, 1988). The enzyme constitutes about 50% of soluble proteins in the plant leaf and is identified as the most abundant protein on earth (Andersson & Backlund, 2008). Despite its abundance in nature, RuBisCO remains underutilized as a protein in industrial food formulations (Kobbi et al., 2017), even after its functionalities and prospective food applications were highlighted three decades ago (Barbeau & Kinsella, 1988). This slow progress is likely due to technological challenges associated with plant RuBisCO extraction or their low yield on a leaf weight basis, since 90% of green leaf is water. Emerging trend is now looking into converting non-edible and food waste vegetables into soluble protein isolates for food applications. These proteins are expected to be comprised mostly of RuBisCO. Apart from direct utilization as dietary source of amino acids in food products, one notable application explored for RuBisCO is its use as a sustainable source of bioactive peptides. As discussed later in this paper, RuBisCO-derived peptides have demonstrated beneficial effects for health promotion *in vitro*, in cultured cells and animal models. Moreover, bioinformatics has revealed several untapped peptides, most of which can be released from the large and small RuBisCO subunits by specific enzymatic proteolysis. These unexplored peptides can play several biological roles for the prevention and management of cardiovascular diseases, diabetes, immune and neurodegenerative diseases. The objective of this review is to discuss the structure, function, composition and technology for plant RuBisCO extraction as well as the fractionation and known bioactivities of its enzymatic hydrolysate and peptides for use in various food and health applications.

2. Structure and function of RuBisCO

RuBisCO is a major enzyme that catalyzes the first step of carbon dioxide fixation in net photosynthesis and carbon oxidation during photorespiration. Its primary role is the conversion of carbon dioxide from the biosphere into organic compounds in the rate-limiting step of the Calvin cycle (Spreitzer & Salvucci, 2002). This is achieved through the carboxylation of ribulose-1,5-bisphosphate to produce two molecules of 3-phosphoglycerate (Andersson & Backlund, 2008). The affinity of RuBisCO for carbon dioxide favours its carbon fixation catalytic activity. However, this is impeded by its affinity for molecular oxygen resulting in photorespiration. This process slows down the catalytic activity of carbon dioxide fixation and decreases the activity by 50%; thus, RuBisCO is often referred to as an inefficient enzyme (Andersson & Backlund, 2008; Spreitzer & Salvucci, 2002). Consequently, photosynthetic organisms produce RuBisCO in large amounts in order to meet their metabolic needs, and it is estimated to make up over 50% of the total amounts of soluble proteins in the plant leaf (Spreitzer & Salvucci, 2002).

RuBisCO is a large multimeric protein with a molecular weight of 560 kDa and is found in all photosynthetic, chlorophyll-containing organisms including higher plants, green and blue-green algae, chemolithotrophic bacteria, green sulphur and purple non-sulphur bacteria (Barbeau & Kinsella, 1988; Kobbi et al., 2017). Form I holoenzymes are the most common form of RuBisCO found in land plants and green algae (Andersson & Backlund, 2008; Spreitzer & Salvucci, 2002). They possess a hexadecameric structure consisting of eight large and eight small subunits, L8S8, which exhibit 422 local symmetry (Andersson & Backlund, 2008). Form II RuBisCO proteins are dimeric, possess two large subunits and lack the small subunits. They are found in some prokaryotes and dinoflagellates including purple non-sulphur and chemoautotrophic bacteria (Andersson & Backlund, 2008; Morse, Salois, Markovic, & Hastings, 1995; Tabita, 1999; Whitney & Andrews, 1998). On the other hand, RuBisCO in archaebacteria consists of a decamer with five large subunit dimers (Maeda et al., 1999).

3. Chemical composition and physicochemical properties of RuBisCO

The composition and sequence of large RuBisCO subunits from different plants are highly conserved, whereas there are notable differences in the small subunits. RuBisCO derived from spinach (*Spinacia oleracea*) and alfalfa (*Medigaco sativa*) have particularly been used to produce bioactive peptides and hydrolysates. The structure of spinach RuBisCO showing the large and small subunits is provided in Fig. 1. The sequence of the large subunits is identical across different plant species. Using UniProtKB Align, the polypeptide chains of the large RuBisCO subunit obtained from spinach, alfalfa and sunflower (*Helianthus annuus*) containing 473, 472 and 483 amino acid residues, respectively, showed 89.5% sequence homology and 434 identical positions. Conversely, the small subunits of plant RuBisCO contain about 123 amino acid residues with less sequence homology; using the example above, 41.6% homology and 75 identical positions were observed for the small chain. RuBisCO has a net negative charge at neutral pH with isoelectric point (pI) of 4.4–4.7, for spinach, maize, cotton and tobacco RuBisCO, and a large charge/mass ratio that promotes its tendency to bind cations (Barbeau & Kinsella, 1988). The protein is hydrophobic with a relative hydrophobicity value of about 1275 cal/residue (Barbeau & Kinsella, 1988). From calculated values of the grand average of hydropathicity (GRAVY), alfalfa RuBisCO large subunit is more hydrophobic than the small subunit due to its higher total hydrophobic amino acid content (Table 1). Native alfalfa RuBisCO contains one free sulfhydryl group per protomer and others localized within the structure in the form of disulphide bridges (Barbeau & Kinsella, 1988). The predominant amino acid residues in alfalfa