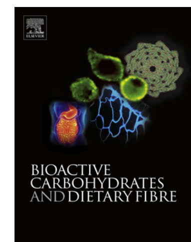
Bioactive Carbohydrates and Dietary Fibre 4 (2014) 27–38

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/bcdf



Inhibition of digestive enzyme activities by pectic polysaccharides in model solutions

*Mauricio Espinal-Ruiz, Fabián Parada-Alfonso, Luz-Patricia Restrepo-Sánchez, Carlos-Eduardo Narváez-Cuenca**

Departamento de Química, Facultad de Ciencias, Universidad Nacional de Colombia, AA 14490 Bogotá DC, Colombia

ARTICLE INFO

Article history:
Received 25 March 2014
Received in revised form
19 June 2014
Accepted 19 June 2014

Keywords:
Pectic polysaccharide
Methylation degree
Digestive enzymes
Hydrophobic interactions
Non-competitive inhibition

ABSTRACT

The presence of dietary fiber (e.g., pectic polysaccharides, PPs) in the gastrointestinal tract may decrease the caloric intake and reduce the risk of developing cardiovascular diseases. These phenomena are governed by several mechanisms, such as the regulation of the rate of nutrient absorption and the alteration of the normal activity of the gastrointestinal tract enzymes. In this study, we evaluated the effect of PPs with five methylation degrees (MD) on the activities of lipase, α-amylase, alkaline phosphatase, and protease. The MD of the PPs ranged (in mol/mol) from 87.4% (high-methylated PP, HMPP) to 7.1% (low-methylated PP, LMPP). The enzymatic activities were evaluated in model solutions after incubation with PPs. The Michaelis–Menten constant remained unmodified whereas the apparent maximum velocity (V_{maxapp}) decreased with increasing PP concentrations. The V_{maxapp} represented 13.3%, 38.6%, 41.9%, and 44.4% of the V_{max} (without PPs) for lipase, α-amylase, alkaline phosphatase, and protease, respectively, when they were inhibited with 100 μg mL^{-1} HMPP. Kinetic analyses showed that all of the tested PPs behaved as non-competitive inhibitors of digestive enzymes. Increasing both the concentration and MD of the PPs reduced the enzymatic activities by decreasing the non-competitive inhibition constant (K_i). In plotting K_i versus MD, a straight line was obtained, with slopes of 1.943, 1.558, 1.344, and 1.165 μg $mL^{-1}\%^{-1}$ for lipase, α-amylase, alkaline phosphatase, and protease, respectively. Among them, lipase was most likely to be inhibited by the PPs. Our results suggested that PPs might be able to suppress digestion by inhibiting digestive enzymes.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The main function of the gastrointestinal tract is the absorption of nutrients derived from food digestion. This function is controlled by a series of digestive processes that occur in different sections of the gastrointestinal tract. These digestive processes are controlled by the secretion of digestive enzymes and their associated cofactors and by the stability of the pH and temperature conditions of the gastrointestinal tract (Dawson, 1993). Lipases, α-amylases, alkaline phosphatases, and proteases are the main gastric and pancreatic enzymes present in the gastrointestinal tract (Rothman, 1977). These enzymes are responsible for the hydrolysis of the triglycerides, carbohydrates, and proteins that are consumed in diet, which are carriers of a great caloric content. It has been postulated that the presence of any type of dietary fiber in the gastrointestinal tract may result in the decrease of the total caloric intake (Amarowicz, Kmita-Glazewska, & Kostyra, 1990). This phenomenon is governed by several mechanisms, such as the regulation of the nutrient

*Corresponding author. Tel.: +57 1 3165000x14458; fax: +57 1 3165220.
E-mail address: cenarvaezc@unal.edu.co (C.-E. Narváez-Cuenca).

http://dx.doi.org/10.1016/j.bcdf.2014.06.003
2212-6198/© 2014 Elsevier Ltd. All rights reserved.

absorption rate, perturbations of the intestinal physiological conditions, the encapsulation of minerals and vitamins needed for metabolic processes, and alterations of the normal activities of the digestive enzymes (Kumar & Chauhan, 2010).

Recent epidemiological studies have shown that the consumption of dietary fiber is associated with the reduction of the risk of developing chronic cardiovascular diseases. Consequently, it has been recommended to increase the intake of products of plant origin that have high dietary fiber levels, particularly soluble dietary fiber, together with other phytochemical constituents (Kris-Etherton et al., 2002). Pectic polysaccharides (PPs) are a type of soluble dietary fiber that cannot be digested in the gastrointestinal tract due to their resistance to the hydrolytic action of digestive enzymes (Holloway, Tasman-Jones, & Maher, 1983). PPs also promote bacterial fermentation in the large intestine, improving the proliferation of intestinal microbiota that is beneficial for human health (Louis, Scott, Duncan, & Flint, 2007).

In the gastrointestinal tract, PPs form a complex three-dimensional matrix with fibrous and amorphous characteristics. Physicochemical properties, such as the methylation degree (MD), acetylation degree, molecular weight distribution, distribution of non-methylated galacturonic acid residues, methylation and acetylation distribution patterns, and gel-forming capacity (Brownlee, 2011), as well as the structure of the three-dimensional matrix, play important roles in the homeostatic and therapeutic functionality of PPs in human nutrition (Kay, 1982). Enhancement of the gastrointestinal viscosity, inhibition of digestion and the absorption of nutrients, control of gastrointestinal motility and immunity, regulation of the activity of the colonic microbiota, and regulation of a systemic stimulus associated with the feeling of satiety are among the therapeutic properties and physiologic effects that are beneficial for human health that have been attributed to PPs (Brownlee, 2011).

Regulation of the gastrointestinal tract enzymatic activity by dietary fiber from different plant materials was previously reported (Dunaif & Schneeman, 1981; Isaksson, Lundquist, & Ihse, 1982a, 1982b; Ikeda & Kusano, 1983; Tsujita et al., 2007). The dietary fiber materials contribute to inhibiting the activity of gastrointestinal tract enzymes. According to the afore cited work, the occurrence of physical interactions (such as ionic interactions, hydrogen bonding, dispersive forces, and hydrophobic interactions) might play an important role in the capacity of the dietary fiber to inhibit enzymatic activities.

However, the experiments conducted in those studies, did not identify a kinetic mechanism underlying the inhibition of such enzymes. Inhibition of the activity of the enzymes by ingested PPs might play an important role in reducing the quantity of free fatty acids, monosaccharides, and amino acids that can be absorbed at the gastrointestinal tract level (Ikeda & Kusano, 1983). Identifying the mechanism by which PPs act as inhibitors of digestive enzymes in model solutions might be useful in understanding the physiological phenomena involved in the *in vivo* regulation that PPs exert on nutrient absorption at the gastrointestinal tract level (Brownlee, 2011).

The aim of this study was, therefore, to evaluate in model solutions the effects of both the concentration and MD of PPs on the activities of lipase, α-amylase, alkaline phosphatase, and protease (chymotrypsin). This study also aimed to determine a kinetic mechanism by which PPs inhibit the activity of enzymes, as well as to evaluate theoretically, through molecular docking calculations, the influence of structural parameters on the enzyme–PP surface interaction.

2. Materials and methods

2.1. Chemicals

The enzymes porcine pancreatic α-amylase, from *Sus scrofa* (16 U mg^{-1} type VI-B, E.C. 3.2.1.1) porcine pancreatic lipase, from *Sus scrofa* (100 U mg^{-1} type II, E.C. 3.1.1.3) bovine pancreatic protease (chymotrypsin), from *Bos taurus* (5 U mg^{-1} type I, E.C. 3.4.21.1), and bovine intestinal mucosa alkaline phosphatase, from *Bos taurus* (10 U mg^{-1} type I, E.C. 3.1.3.1); the artificial substrates 2-chloro-*p*-nitrophenyl-α-D-maltotrioside (G_3CNP), *p*-nitrophenyl palmitate (*p*NPPA), *p*-nitrophenyl acetate (*p*NPA), and *p*-nitrophenyl phosphate (*p*NPP); the reaction products *p*-nitrophenol (*p*NP) and 2-chloro-*p*-nitrophenol (CNP); and the protein determination reagent brilliant blue G-250 (Coomassie Blue); as well as bovine serum albumin were purchased from Sigma-Aldrich (St. Louis, MO, USA). A high-methylated citrus pectic polysaccharide (HMPP) was purchased from CIMPA (Bogotá, Colombia). Ox bile extract with cholic acid content higher than 55% (w/w) was purchased from MP Biomedicals (Solon, OH, USA). Other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Pectic materials

2.2.1. Preparation of pectic polysaccharides

Alkaline de-esterification of the HMPP was performed as described by Dongowski (1997) to obtain PPs with different MD levels. One gram of HMPP was mixed with 50 mL of 0.25 M NaOH (pH of the mixture $\geq$ 10.0) and stirred for 0, 9, 15, 30, or 45 min at 25 °C. The mixture was neutralized to pH 7.0 with 3 M HCl and then 150 mL of 80% (v/v) ethanol were added to induce PP precipitation. The partially de-esterified pectic materials were filtered and washed with 100 mL of 80% (v/v) ethanol. The PPs were dried at 70 °C for 5 h and then the MD, total uronic acid content, acetylation degree, and molecular weight distribution were evaluated.

2.2.2. Characterization of the pectic polysaccharides

2.2.2.1. Determination of total uronic acid content. The total uronic acid content was determined according to van den Hoogen et al. (1998). An aliquot of 400 μL of each PP solution (100 μg mL^{-1}) was mixed with 2 mL of concentrated sulfuric acid (98%, w/w) containing 120 mM sodium tetraborate and incubated for 60 min at 80 °C. After cooling to room temperature, the background absorbance of the samples was measured at 540 nm. Then, 400 μL of *m*-hydroxydiphenyl reagent (100 μL of 100 mg mL^{-1} *m*-hydroxydiphenyl in dimethyl sulfoxide, mixed with 4.9 mL of 80% (v/v) sulfuric acid) was added and mixed with the samples. After 15 min, the absorbance of the pink-colored samples was measured at 540 nm. A calibration line was obtained using galacturonic acid at

Download English Version:

https://daneshyari.com/en/article/1413035

Download Persian Version:

https://daneshyari.com/article/1413035

Daneshyari.com