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Cellulosic fraction of rice bran fibre alters the conformation and inhibits the activity of porcine pancreatic lipase

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ABSTRACT

A cellulosic rice bran insoluble dietary fibre (RBIDF) with different structures and physicochemical properties is shown to inhibit the activity of pancreatic lipase (PL) at different levels. The adsorption rate and saturation level to PL were greatest for RBIDF-2.0 (modified with 2.0% H_2SO_4 + 1.25% KOH), which had the highest specific surface area and oil holding capacity. The conformational deformation of unbound PL in the supernatant of PL-RBIDF confirmed by circular dichroism (CD) suggested that not only bound but also unbound PL activities were altered. Free energy calculations using data from fluorescence spectroscopy revealed that binding of PL to fibre depends primarily on electrostatic interactions, and the binding process is spontaneous and exothermic. This study indicates that dietary intake of cellulosic fractions of rice bran may be useful in reducing the bioavailability of dietary fat by altering the conformation and activity of PL that might be useful to control obesity.

1. Introduction

Obesity is still a serious and an unsolved global health problem, which often leads to type 2 diabetes, despite that considerable research on obesity and type 2 diabetes continue to increase (Ginter & Simko, 2012). Intake of diets high in fat, not only high in calories but also low in fibre, is implicated in obesity related dietary disorders (Steyn et al., 2004). Reducing the bioavailability of dietary fat by inhibiting digestive lipases has been an approach to reduce obesity (Carrière et al., 2001). Pancreatic lipase (PL) is the principal lipolytic enzyme responsible for the hydrolysis of 50–70% total dietary fats (Bhutani, Birari, & Kapat, 2007). Thus, inhibiting the activity of PL could be an effective strategy for preventing systemic absorption of lipids.

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Chemical compounds: Sulfuric acid (PubChem CID: 1118); Potassium hydroxide (PubChem CID: 14797); Sodium hydroxide (PubChem CID: 14798); Monosodium phosphate (PubChem CID: 23672064); Disodium hydrogen phosphate (PubChem CID: 24203); Porcine pancreatic lipase (PubChem CID: 54603431); *n*-hexane (PubChem CID: 8058); polyvinyl alcohol (PubChem CID: 11199); phenolphthalein (PubChem CID: 4764).

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The activity of PL is affected by food constituents such as citrus flavonoid (Srinivasan & Pari, 2013), litchi flower–water extracts (Wu et al., 2013), lignin (Zhang, Xiao, Yang, Wang, & Li, 2014a) and dietary fibres (Isaksson, Lundquist, & Ihse, 1982; Lairon et al., 1985), suggesting that the higher intake of dietary fibres may not only reduce caloric density but may be also related to digestibility of fat. The hypocholesterolemic and hypotriacylglyceroldaemic effects of fibres, including pectin, wheat bran and cellulose, have been repeatedly observed in laboratory animals (Vahouny et al., 1980), but the capacity of wheat bran, a mainly insoluble fibre, to reduce absorption of cholesterol and triglycerides was marginal. However, oat bran, wheat fibre, and wheat germ significantly reduced and rice bran tended to reduce postprandial lipaemia in healthy adults (Cara et al., 1992). The possible mechanism may be due to partial inhibition of gastrointestinal lipases (Borel, Lairon, Senft, Chautan, & Lafont, 1989; Isaksson et al., 1982; Lairon et al., 1985; Schneeman, 1978). These studies suggest that reducing intestinal absorption of fat and cholesterol is related to the nature of the fibre source (Cara et al., 1992).

Rice bran has been shown to have a hypocholesterolaemic effect in rats (Topping et al., 1990) and some beneficial effects in mildly hypercholesterolaemic men (Kestin, Moss, Cliftonm, & Nestel, 1990), but has not been well studied as a potential PL inhibitor. Schneeman (1978) showed that rice bran reduced lipase activity, which was due in part to the binding of the enzyme to the fibre matrix. However, most previous research evaluated the effects of whole rice bran on lipase activity rather than the fibre isolates enriched in cellulose.

China is the world's largest producer of rice (http://www.fas.usda.gov/psdonline/psdHome.aspx), contributing to the global production of 70 million tonnes of rice bran annually (FAOSTAT, 2012). Rice bran has a high content, typically >20%, of insoluble fibre. The insoluble fibre consists of approximately equal amounts of cellulose and hemicellulose. The binding capacity of hemicelluloses to bile acids *in vitro* and *in vivo* (Normand, Ory, & Mod, 1987) may play a role in lowering serum cholesterol. Bile acids are also required to micellarize fat for hydrolysis by lipases. The effects of other cereal brans on lipase activity have been reported (Isaksson et al., 1982; Lairon et al., 1985). However, the mechanism by which bran decreases pancreatic lipase activity still remains unknown. To our knowledge, there are no previous reports that describe the effects of isolated rice bran insoluble dietary fibre (RBIDF) on lipase activity. In this study RBIDF was prepared by treatment of defatted rice bran with 0.2, 1.25 and 2.0% (w/v) H_2SO_4 followed by 1.25% KOH, respectively. The effects of the modified RBIDFs on pancreatic lipase activity using emulsified olive oil as a substrate were evaluated *in vitro*. The lipase adsorption capacities of the modified RBIDFs were compared, and the mechanism of interaction between PL and different RBIDFs was investigated or confirmed by fluorescence spectroscopy and circular dichroism (CD).

2. Materials and methods

2.1. Materials

Fresh paddy rice bran was obtained from DaYang Rice Company (Wuxi, Jiangsu Province, China). Porcine pancreatic lipase (PL, L3126, type II from porcine pancreas) was purchased from Sigma (St Louis, MO, USA). Sodium phosphate buffer (0.1 M NaH_2PO_4–Na_2HPO_4 solution, pH 7.2) was used for all experiments. All other reagents used were of analytical grade.

2.2. Modification of rice bran insoluble dietary fibre (RBIDF)

The rice bran was screened through a 40 mesh sieve and defatted with *n*-hexane (1:5, w/v). The defatted rice bran was heated in 0.2, 1.25 and 2.0% (w/v) H_2SO_4 solutions at 100 °C, separately, at a 1:10 ratio for 30 min, followed by 1.25% KOH solution for 30 min at 100 °C, and then filtered and washed with hot water. The three different RBIDF preparations were designated as RBIDF-0.2, RBIDF-1.25, and RBIDF-2.0, separately. The resultant sample was dried at 60 °C for 24 h, then ground using a high-speed universal grinder (DFY-200, Linda machinery Co., Zhejiang, China) to pass through a 0.25 mm sieve and stored in a desiccator prior to use.

2.3. Characterization of RBIDF

Crude protein was determined by the Kjeldahl method (AOAC, 2005), with a conversion factor of 6.25. Starch content was based on the monosaccharide method using 0.9 as the conversion factor (AOAC, 2005). The water soluble dietary fibre (WSDF) content was measured based on the method of Abdul-Hamid and Luan (2000) with some modifications. The determination was conducted by soaking the fibre into water in an ultrasonic water bath for 1 h without enzyme hydrolysis. The mixture was filtered and the filtrate was precipitated with 95% ethanol (preheated to 60 °C). Hemicellulose and cellulose contents were determined according to the procedure described by Sun, Sun, Tomkinson, and Baird (2003) and Egüés, Sanchez, Mondragon, and Labidi (2012). The water holding capacity (WHC) was determined according to the method of Robertson et al. (2000) and the measurement for oil binding capacity (OBC) was conducted based on the method of Sangnark and Noomhorm (2003). The Brunauer–Emmett–Teller (BET) method was used to calculate the specific surface area of the fibre using the specific surface area and porosimetry analyzer (ASAP 2020 MP; Micromeritics Instrument Corp., Atlanta, GA, USA).

2.4. Lipase adsorption capacities of different RBIDFs

The adsorption under a range of PL concentrations (0–2.2 mg/L) by RBIDF over a period of 0–2 h was determined. Briefly, 5 mL of PL solution (prepared with 0.1 M, pH 7.2 phosphate buffer) was mixed with 0.3 g of RBIDF (RBIDF-0.2, -1.25 or -2.0) and was magnetically stirred at 37 °C for various times. The mixture was filtered using a nylon mesh after a fixed period of time and the filtrate was collected. The RBIDF residue with adsorbed enzyme was washed three times using 2 mL of the same buffer. The protein content of the enzyme solution before and after adsorption was measured by the method of Bradford (1976) using bovine serum albumin as a standard. The amount of adsorbed lipase protein was estimated by subtracting the amount of protein determined in the filtrate and washings from the total amount of protein used in the adsorption procedure. Lipase