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Phytonutrients for controlling starch digestion: Evaluation of grape skin extract

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ABSTRACT

The objective of this work was to evaluate the structure–function relationship between grape skin extract and human α-amylase. The grape skin extract was characterised as resveratrol-3-*O*-glucoside by RP-HPLC–ESI-MS, which showed strong inhibition towards α-amylase and the IC_{50} value was 1.35 mg/ml. The kinetic results demonstrated grape skin extract obeyed the non-competitive mode against amylase. Fluorescence data revealed the ability of grape skin binding to amylase belonged to static quenching mechanism with a complex formation and there was only one binding site in α-amylase for grape skin extract. Docking study showed a best pose with total energy value of −118.3 kJ/mol and grape skin extract interacted with side chain of Asp300 with hydrogen bonds and Van der Waals forces. This preliminary observation provides the basis for further evaluation of the suitability of grape skin extract as natural inhibitor with potential health benefits.

1. Introduction

Diabetes mellitus and obesity have become major public health concerns worldwide, with the number of cases increasing exponentially in recent years. The multi-factorial aetiology of this worldwide epidemic, and the idea that dietary factor may contribute to it, is now well recognised (Aston, 2006; Coll, Farooqi, & O'Rahilly, 2007; Englyst & Englyst, 2005; Sies, Stahl, & Sevanian, 2005). Compelling evidence from epidemiologic studies indicates that the blood glucose from ingested carbohydrate sources is necessary to reduce the complications and cost for controlling and preventing the metabolic syndromes, including diabetes and pre-diabetes, cardiovascular diseases, obesity and overweight (Ludwig, 2002; Semjonous et al., 2009; Sies et al., 2005). New developments in food and nutritional science have led to the conclusion that slowing down the rate of carbohydrate digestion helps to blunt glycaemia, reduces insulin requirements, and causes satiety by reducing the stress on regulatory systems related to glucose homeostasis and energy metabolism (Aston, 2006; Coll, Farooqi, & O'Rahilly, 2007; Englyst & Englyst, 2005; Miao, Jiang, & Zhang, 2009; Semjonous et al., 2009; Zhang & Hamaker, 2009).

Amongst food carbohydrates, starch occupies a unique position based on the basic source of metabolic energy for the majority of the world's population. According to the rate and extent of digestibility and the corresponding postprandial glycaemic response, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) related to its physiological effect after consumption (Englyst, Kingman, & Cummings, 1992). RDS is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine leading to a fast elevation of blood glucose and insulin level, and RS is not digested in the upper gastrointestinal tract, but its microbial fermentation in the colon produces short chain fatty acids (SCFA) that is beneficial to colonic health, whilst SDS is digested slowly throughout the entire small intestine to provide sustained glucose release with a low initial glycaemia and subsequently a slow and prolonged release of glucose, which is essential to regular physiological processes and optimal health (Englyst & Englyst, 2005). Therefore, improving food quality with higher amounts of SDS is becoming a hot research filed for researchers from industry and academia. There are numerous reports and patents on SDS preparation, but there is no commercially available SDS or SDS-state foods in the market (Miao, Zhang, Mu, & Jiang, 2010; Miao et al., 2009; Zhang & Hamaker, 2009).

In the past decades, considerable research effort has been devoted to novel ways for achieving the physiological effects of SDS for glycaemic control and the prevention of related diseases (Björck, Liljeberg, & Östman, 2000; Ludwig, 2002). Compared to the commercial inhibitors (i.e. Acarbose and Phase II), the interactions of starch with different components present in the food system have become an innovative target for improvement of

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postprandial hyperglycaemia with fewer gastrointestinal side effects (Obiro, Zhang, & Jiang, 2008; Sies et al., 2005). Besides two most important interactions between starch and protein or lipid, the soluble fibres (guar gum, psyllium, β-glucan or pectin), antinutrients (tannin, phytate, saponin or lectin), phenolic compounds (luteolin, myricetin or quercetin) and organic acids (lactic acid, propionate or vinegar) have been used to improve overall glycaemic control by inhibition of amylolytic enzymes (Gonçalves, Mateus, & de Freitas, 2011; McDougall, Kulkarni, & Stewart, 2008; Zhang & Hamaker, 2009). Recent studies have shown that grape is a natural source of notable bioactive compounds, such as flavonols, anthocyanins and procyanidins, which are can positively influence risk factors associated with cardiovascular health, cancer, diabetes, inflammation, neurodegenerative disease, and age-related cognitive decline (Chuang et al., 2012; Hogan et al., 2011; Vislocky & Fernandez, 2010; Zunino, 2009). Also, resveratrol is a unique component of grapes and has anti-aging, anti-carcinogenic, anti-inflammatory, and anti-oxidant properties that might be relevant to chronic diseases and/or longevity in humans (Smoliga, Baur, & Hausenblas, 2011). In particular, the stilbene resveratrol has shown potential for reducing hyperglycaemia, improving insulin sensitivity, and protecting against β-cell loss (Brasnyó et al., 2011; Lagouge et al., 2006; Zunino, 2009). However, very little information exists regarding grape skin extract related to control starch digestion. In the current investigation, the structure–function relationship between grape skin extract and human α-amylase was elucidated with *in vitro* assays and *in silico* modelling, which is important for practical application in making tailor-made carbohydrate foods with low glycaemic index.

2. Materials and methods

2.1. Materials

The grape skin extract sample was purchased from Riotto Botanicals Co., Ltd. (Shaanxi, China). Normal maize starch was obtained from Changchun Dacheng Industrial Group Co. Ltd. (Changchun, Jilin, China). Alpha-amylase (Cat. No. A-9972, ⩾100 units/mg protein) from human pancreas and piceid (Cat. No. 15721, ⩾95%) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). All chemicals were reagent grade and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Polyphenols assays

The grape skin extract sample (approximate 1 mg) was dissolved in 10 ml of methanol and then centrifuged (5000*g* for 10 min). The supernatant was filtered through a 0.22 μm membrane filter and then injected into a Waters 2690 HPLC with a Micromass ZMD mass spectrometer, and a Waters 996 diode array detector (Waters Corp., Milford, MA, USA). The acquisition and processing data were performed by the version 3.1 MassLynx software. An analytical reverse phase C18 column (250 × 4.6 mm, Purospher® STAR, 5 μm, Merck Millipore International) was used in the analysis. The mobile phase was composed of 1% acetic acid in water (v/v, A) and methanol (B). The linear gradient conditions were as follows: from 0% to 100% B in 40 min flow at a flow rate of 0.3 ml/min and the column temperature set at 35 °C. The polyphenol was detected by monitoring the elution at 520 nm. The electrospray ionization mass spectrometry parameters were as follows: positive mode, capillary voltage 3.8 kV, cone voltage 30 V, extractor voltage 5 V, source block temperature 120 °C, desolvation temperature 300 °C and gas flow of N_2 9 L/min, nebulizer pressure 60 psi, scan range from m/z 100–900 with scan time 1 s and interscan delay 0.1 s.

The resveratrol derivative was analysed using an Agilent 1100 HPLC system (Agilent Technologies, USA) equipped with a reversed phase symmetry C18 column (250 × 4.6 mm, Waters, USA). The chromatographic conditions were as follows: mobile phase composed solvent A (10% water, v/v) and solvent B (60% acetonitrile, v/v); injection volume 5 μl; flow rate 1.0 ml/min; and quantification of resveratrol at 304 nm. The piceid was used as a standard sample for HPLC test.

2.3. Alpha-amylase assays

The digestibility of each starch was analysed according to the procedure of Englyst et al. (1992) with a slight modification. Enzyme solution was prepared by suspending human pancreatic α-amylase (12.0 g) in phosphate buffer (100 ml, 0.2 M, pH 5.2) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500 g, and then transferring a portion (50 ml) of the supernatant into a beaker. The maize starch sample (200 mg) was dissolved in 15 ml of phosphate buffer (0.2 M, pH 5.2) by heating at 95 °C for 10 min. After the solution was cooled and equilibrated at 37 °C for 5 min, enzyme solution (5.0 ml) and grape skin extract (10%, based on starch) were added. Then, the samples were shaken in a 37 °C water bath at 150 rpm. Aliquots of hydrolysed solution (0.5 ml) were taken at different time intervals and mixed with 4 ml of absolute ethanol to deactivate the enzymes. The reducing sugar content was determined with the Nelson-Somogyi method by measuring the absorbance at 540 nm. A control vial was prepared by replacing the inhibitor solution with phosphate buffer. Percentage of pancreatic α-amylase inhibition was calculated according to the equation below:

$$\%\text{Inhibition} = \frac{(A_{\text{control}} - A_{\text{controlblank}}) - (A_{\text{sample}} - A_{\text{sampleblank}})}{A_{\text{control}} - A_{\text{controlblank}}} \times 100$$

where A_{control}, $A_{\text{controlblank}}$, A_{sample} and $A_{\text{sampleblank}}$ refer to the absorbance value of reaction vial containing live enzyme and buffer, dead enzyme and buffer, live enzyme and inhibitor and dead enzyme and inhibitor respectively. Substrate was present in all these vials. IC_{50} value (concentration of inhibitor required to produce a 50% inhibition of the initial rate of reaction, mg/ml) was obtained graphically by an inhibition curve.

The Michaelis–Menten kinetic model was employed to evaluate the effect of grape skin extract on starch hydrolysis. The amount of glucose liberated under different starch concentrations (5, 10, 15, 20, 25 mg/ml) of cooked maize starch in the presence of grape skin extract (0, 1, 4 mg/ml) was used to measure the type of inhibition. A Lineweaver–Burk plot between 1/[*S*] (starch concentration) and 1/[*V*] (reaction rate) was used to examine the action type of grape skin extract on the starch hydrolysis.

2.4. Fluorescence measurements

The quenching effect of grape skin extract on human α-amylase fluorescence was assayed as described in the literature with some modification (Lakowicz, 2006). A HITACHI fluorescence spectrometer (Model 650–60, Hitachi, Tokyo, Japan) was used for Fluorescence quenching assays. The sample (1 ml) was excited at 280 nm, with 1 nm excitation and emission slits, and spectra were recorded between 300 and 500 nm at 0.1 nm resolution. A stock solution of α-amylase and the quenchers of grape skin extract were prepared by dissolving in phosphate buffer (pH 5.2). The fluorescence intensities were obtained at different grape skin extract concentrations and plotted according to the Stern–Volmer equation. The quenching constant K_{sv}, the quenching rate constant K_q, the number of binding sites n and apparent associative binding constant K_a were obtained using the slopes and intercept of these