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Ability of resveratrol to inhibit advanced glycation end product formation and carbohydrate-hydrolyzing enzyme activity, and to conjugate methylglyoxal

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ABSTRACT

Glycation can generate advanced glycation end products (AGE) and its intermediates methylglyoxal (MGO) and glyoxal in foods, which increase the risk of developing diabetes diseases. In this study, the effect of resveratrol against AGE formation, carbohydrate-hydrolyzing enzyme activity and trapping MGO capability were evaluated. Resveratrol showed a significant inhibition capability against AGE formation in bovine serum albumin (BSA)-fructose, BSA-MGO and arginine-MGO models with inhibition percentages of 57.94, 85.95 and 99.35%, respectively. Furthermore, resveratrol acted as a competitive inhibitor for α-amylase with IC_{50} 3.62 μg/ml, while it behaved in an uncompetitive manner for α-glucosidase with an IC_{50} of 17.54 μg/l. A prevention of BSA protein glycation was observed in the BSA-fructose model with addition of resveratrol. Three types of resveratrol-MGO adducts were identified in the model consisting of MGO and resveratrol. The results demonstrated that resveratrol has potential in reducing glycation in foods and retarding carbohydrate-hydrolyzing enzyme activities.

1. Introduction

Glycation is a reaction between reducing sugars and the free amino residues of proteins without enzyme intervention (Nguyen, Pascariu, & Ghitescu, 2006). The sugar-peptide adducts undergo oxidation or fragmentation and evolve gradually towards a population of heterogeneous macromolecules, characterized as "advanced glycation end products" (AGEs) (Rojas & Morales, 2004). Typical reactive dicarbonyl species such as glyoxal, and methylglyoxal (MGO) are the primary intermediates that form AGEs in both foods and human body. In biological systems, serum proteins exposed to high concentrations of reducing sugars are susceptible to glycation as a post-translational modification assisted by the reactive dicarbonyl species (Ledesma-Osuna, Ramos-Clamont, & Vazquez-Moreno, 2008). The glycated proteins are then altered in molecular weight and structure, containing highly reactive free radicals and extensive intra or intermolecular cross-links, which are involved in many biochemical abnormalities associated with ageing and diabetes (Ledesma-Osuna et al., 2008).

On the other hand, carbohydrate-hydrolyzing enzymes including α-amylase and α-glucosidase are involved in catalyzing the hydrolysis of starch to form glucose in the digestive system and are responsible for postprandial hyperglycemia (Thilagam, Parimaladevi, Kumarappan, & Mandal, 2013). In order to control glycation related diseases, therapeutic approaches could be developed based on the mechanisms of antiglycation by conjugating dicarbonyl species or limiting of glucose release by inhibiting α-amylase and α-glucosidase. Currently, synthetic medicines such as aminoguanidine, metformin, pioglitazone and aspirin etc, have been clinically used as anti-type 2 diabetes drugs (Sadowska-Bartosz & Bartosz, 2015). However, long-term intake of those medicines would cause nonspecific and potentially toxic effects (Campbell, 1996). Currently, the flavonoids (genistein, quercetin, epicatechin, luteolin, resorinol and phloroglucinol) and phenolic acids (cinnamic, isoferulic, ellagic and chlorogenic acids) from plants have been investigated as natural inhibitors against glycation. They could be potential alternatives to synthetic drugs in the treatment of diabetes or other glycation related diseases (Sadowska-Bartosz & Bartosz, 2015).

Resveratrol (3,5,4-trihydroxystilbene) is a stilbene, belonging to a subclass of phytoalexins and synthesized in response to pathogens and abiotic stress in plants (Shanmuganathan & Li, 2009). It is abundant in low bush berries and grapes. Its antioxidant,

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anti-inflammatory, anticancer and phytoestrogenic effect, as well as inhibition of platelet aggregation have been widely reported (Wang et al., 2005). However, the potential activity of resveratrol against glycation has not been documented yet. In this study, the antiglycation capabilities of resveratrol at different concentrations were evaluated through bovine serum albumin (BSA)-fructose, BSA-MGO and arginine-MGO models. Also, the kinetics of resveratrol in inhibiting α-amylase and α-glucosidase were investigated. In order to understand the antiglycation mechanism of resveratrol, the formations and structures of resveratrol carbonyl conjugate and glycated proteins were monitored by liquid chromatography–tandem mass spectrometry (LC-MS/MS) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) methods. The results of this study provide useful information on antiglycation capability and mechanism of resveratrol. It could be a potential natural compound used as a functional ingredient in food products to reduce AGEs formation or prevent hyperglycemia in diabetes treatment.

2. Materials and methods

2.1. Chemicals and materials

Resveratrol standard, o-phenylenediamine, methylglyoxal (MGO), arginine, bovine serum albumin (BSA), maltose, fructose, aminoguanidine, potassium phosphate dibasic, potassium phosphate monobasic, sodium carbonate, trichloroacetic acid, β-mercaptoethanol, bromophenol blue, glycerol, Tris-HCl buffer, α-glucosidase (from *Bacillus stearothermophilus*), α-amylase (from porcine pancrease), 3,5-dinitrosalicylicacid, 4-nitrophenyl-α-D-glucopyranoside and p-nitrophenyl glucopyranoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol, acetic acid, and acetone, as well as sodium dodecyl sulfate (SDS), and Coomassie brilliant blue R-250 were obtained from Thermo Fisher Scientific Co. (Pittsburg, PA, USA).

2.2. Determination of antiglycation capability by using BSA-fructose, BSA-MGO and arginine–MGO models

A BSA-fructose model was used to monitor the process of BSA protein glycation based on a study by Wang, Yagiz, Buran, Nunes, and Gu (2011) with minor modifications. Fructose (1.5 mol/l) or BSA (60 mg/ml) solution was prepared in potassium phosphate buffer (0.2 mol/l, pH 7.4, containing 0.06% sodium azide for preventing microbial growth), respectively. The BSA (500 μl) and fructose (500 μl) solutions were incubated with 500 μl of resveratrol solution (50, 100, 150, 200, or 300 μg/ml) in a 10 ml test tube with screw cap at 50 °C for 24 h. Deionized water (500 μl) and aminoguanidine (500 μl) (50, 100, 150, 200, or 300 μg/ml) were used to replace the resveratrol solution as a blank and a positive control, respectively, at the same concentration as each resveratrol treatment. The fluorescence intensity of each reaction solution at an excitation wavelength of 360 nm and an emission wavelength of 460 nm was recorded by an F-4500 luminescence spectrometer (Shimadzu, Japan) and used to calculate the formation of AGEs. Antiglycation or inhibition percentage was calculated and expressed using the equation below:

$$\text{Inhibition}\ (\%) = [1 - (\text{intensity of test sample}/\text{intensity of control})] \times 100\%$$

The BSA-MGO model was employed to evaluate the middle stage of protein glycation (Wang et al., 2011). MGO (60 mmol/l), BSA (60 mg/ml), a series of concentrations of resveratrol or aminoguanidine (50, 100, 150, 200, and 300 mg/ml) solutions were prepared in buffer solution (0.2 mol/l, pH 7.4, containing 0.02% sodium azide for preventing microbial growth). Then, 500 μl of the MGO solution was mixed with 500 μl of each resveratrol solution in a 10 ml test tube with screw cap. After the mixture was incubated at 37 °C for 2 h, 500 μl of the BSA solution was added and incubated at 37 °C for six days. Deionized water (500 μl) and aminoguanidine (500 μl) were used to replace the resveratrol solution as a blank and a positive control at the same concentration of each resveratrol treatment, respectively. The fluorescence intensity of each reaction solution at an excitation wavelength of 360 nm and emission wavelength of 460 nm was measured by an F-4500 luminescence spectrometer (Shimadzu, Japan) to calculate the inhibition percentage. The calculation used was the same equation as for the BSA-fructose model.

The arginine-MGO model was used to evaluate the mechanism of AGE production (Wang et al., 2011). MGO (60 mmol/l), arginine (60 mg/ml) or a series of concentrations of resveratrol or aminoguanidine (50, 100, 150, 200, and 300 mg/ml) solutions were prepared in potassium phosphate buffer (0.2 M, pH 7.4, containing 0.02% sodium azide preventing microbial growth). Then, 500 μl of MGO solution was mixed with 500 μl of each resveratrol solution in a 10 ml test tube with screw cap. After the mixture was incubated at 37 °C for 2 h, 500 μl of arginine solution was added and incubated at 37 °C for six days. Deionized water (500 μl) and aminoguanidine (500 μl) were used to replace the resveratrol solution as a blank and a positive control at the same concentration of each resveratrol treatment, respectively. The fluorescence intensity of each reaction solution and the inhibition percentages were determined in the same way as for the BSA-fructose model.

2.3. Determination of inhibitory capability and kinetics against α-amylase and α-glucosidase

The inhibitory capability for α-amylase was conducted based on the procedure of Sheng et al. (2014). An aliquot of 500 μl of resveratrol solution (0.125, 0.25, 0.5, 1.0, 2.0, 4.0, or 6.0 μg/ml) was mixed with 40 μl α-amylase solution (10 mg/ml, 50 U/mg) and 500 μl of 20 mmol/l sodium phosphate buffer (pH 6.9). After being pre-incubated at 37 °C for 10 min, the mixture was mixed with 40 μl of 1% starch solution in sodium phosphate buffer (pH 6.9, 0.02 mol/l) and incubation for another 10 min. The reaction was terminated by adding 20 μl of dinitrosalicylic acid reagent (1%) followed by incubation in boiling water for 10 min. After it was cooled to room temperature, the absorbance of the reaction mixture was measured at 595 nm using a spectrophotometer (UV-1800, Shimazu, Japan). Distilled water instead of resveratrol solution, following the same procedure, was used as a blank. The α-amylase inhibitory capability was calculated as percentage of inhibition:

$$\%\text{Inhibition} = \left[\frac{\text{Abs}_{\text{blank}} - \text{Abs}_{\text{test sample}}}{\text{Abs}_{\text{blank}}}\right] \times 100$$

where $\text{Abs}_{\text{blank}}$ is the absorbance of reaction solution with distilled water and $\text{Abs}_{\text{test sample}}$ is the absorbance of reaction solution with addition of resveratrol.

In the kinetics study, two levels of resveratrol standard solutions (2 and 6 μg/ml) and a control without resveratrol but distilled water were used. The resveratrol capability of inhibiting α-amylase is based on the amount of reducing sugars released from starch, which was determined by the spectrophotometer mentioned above. A calibration curve at 10.28, 14.40, 18.52, 22.63, 26.75, 30.86, 34.98 mmol/l of maltose substrate was set up and used to calculate the reaction velocities. A Lineweaver-Burk reciprocal plot (1/V versus 1/[*S*]) where V and [*S*] are reaction velocity and substrate concentration, respectively, was used to determine the inhibition kinetics of resveratrol against