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Protective effects of cyanidin-3-rutinoside against monosaccharides-induced protein glycation and oxidation

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ABSTRACT

Cyanidin-3-rutinoside (C3R), a naturally occurring anthocyanin, is present in various fruits and vegetables as a colorant. C3R has been well characterized and demonstrated a number of biological activities attributed to its antioxidant properties. The present study compared the effectiveness of C3R against monosaccharide-induced protein glycation and oxidation *in vitro* using bovine serum albumin (BSA).The results demonstrated that C3R (0.125–1.00 mM) inhibited the formation of fluorescent AGEs in ribose-glycated BSA (2–52%), fructose-glycated BSA (81–93%), glucose-glycated BSA (30–74%) and galactose-glycated BSA (6-79%).Correspondingly, C3R (1.00 mM) decreased the level of N^{ε}-(carboxymethyl) lysine (56-86%) in monosaccharide-induced glycation in BSA. C3R also reduced the level of fructosamine, β-amyloid cross structure, protein carbonyl content as well as the depletion of thiol in BSA/monosaccharide system. In summary, C3R might offer a new promising antiglycation agent for the prevention of diabetic complications by inhibiting AGE formation and oxidation-dependent protein damage.

1. Introduction

The rapid growing burden of diabetes has become a serious global public health issue. The prevalence of diabetes for all age-groups worldwide has been estimated to be 2.8% in 2000 and 4.4% in 2030 [1]. It is now well recognized that in both type 1 and type 2 diabetes chronic hyperglycemia plays an important role in the pathology of vascular damage [2,3]. Its deleterious effects are attributable to the formation of sugar-derived substances called advanced glycation end products (AGEs). AGEs result from a non-enzymatic glycation reaction between the carbonyl group of reducing monosaccharides and the free amino group of proteins, commonly described as protein glycation [4,5]. Protein glycation is particularly known to underlie the macro and microvascular complications in diabetes.

The receptor for AGEs (RAGE), first described as a signal transduction receptor for AGEs, has now been identified as a multi-ligand receptor that is able to bind a number of agents including AGEs, high mobility group protein (B)1, S-100 calcium-binding protein, phosphatidyl serine as well as amyloid-β-protein and β-sheet fibrils [6–8]. The interaction of AGEs with RAGE triggers the overproduction of free radicals and promotes inflammation through activation of the MAPK pathway leading to diabetic complications [8]. Indeed, emerging evidence reveals that increased serum level of AGEs is well correlated with the development of vascular complications in type 2 diabetic patients [9,10].

There has been considerable interest in the types of dietary monosaccharides for induction of protein glycation. An aldohexose (glucose and galactose) as well as a ketohexose (fructose) are a type of simple monosaccharide found in plants, fruits, and foods. Aside from hexose sugars that play a vital nutritional role, an aldopentose (ribose) can be obtained from diet, especially from foods containing high content of riboflavin such as cereal, meat and dark-green vegetable. In addition, ribose is endogenously synthesized from glucose through the hexose monophosphate shunt [11]. The rate of protein glycation-mediated by monosaccharides varies in their reactiveness which depends on the

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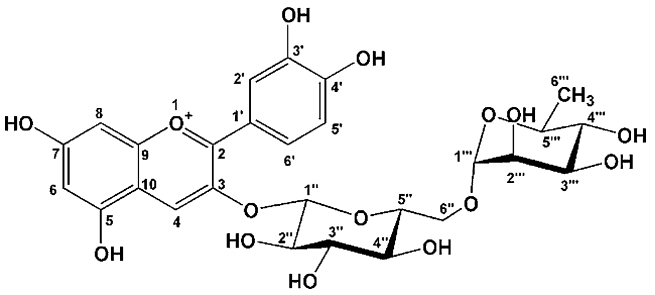

Fig. 1. The chemical structure of cyanidin-3-rutinoside (C3R).

concentration and the conformation of sugar [12,13]. Ribose has been reported to be the most reactive to trigger the glycation process and the formation of AGEs both inside and outside the cells, leading to dysfunction of organs and cognitive impairments [11]. The ribose-glycated bovine serum albumin (BSA) affects to induce protein misfolding and forms globular amyloid-like aggregation, resulting in neuronal death [14]. It has been shown that long-term fructose consumption increases the level of glycated hemoglobin and causes skin collagen crosslinking and collagen-linked glycation in rats [15]. Moreover, non-enzymatic glycation of lens proteins by galactose causes protein aggregation, leading to cataract formation in diabetic patient [16].

Nowadays, there is interest in agents with anti-glycation activity that have been a key strategy for prevention and amelioration of AGE-mediated diabetic complications. Current studies attempt to search for effective phytochemical compounds from dietary plants, fruits, and herbal medicines to inhibit AGEs formation [17–19]. Cyanidin belong to the anthocyanins that are largely distributed in human diet group through crops, vegetables, fruits, and red wine. Previous studies have revealed that a glycosylated form of cyanidin, called cyanidin-3-glucoside, potentially inhibited the formation of AGEs in models of glycated albumin [20]. Cyanidin-3-rutinoside (C3R), a common glycosylated structure, displays a wide range of biological activities including antioxidant, anti-inflammation and anti-carcinogenic [21,22]. Our previous investigation has shown anti-hyperglycemic activity by inhibiting α-glucosidase (maltase and sucrase) and pancreatic α-amylase in vitro and in vivo [23,24]. However, no research has evaluated the potential effectiveness of C3R in relation to its activity against monosaccharide-induced protein glycation in vitro. Therefore, the purpose of this study was to examine antiglycation activity of C3R against monosaccharide-induced protein glycation in vitro, using BSA as the protein source. In addition, the effect of C3R on protein oxidation mediated by glycation was also investigated.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) fraction V was purchased from Fisher scientific (Hudson, NH, USA). 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), thioflavin T, nitrobluetetrazolium (NBT), 1-deoxy-1-morpholino-D-fructose (DMF), L-cysteine, and aminoguanidine hydrochloride (AG) were purchased from Sigma (St. Louis, MO, USA). 2,4-dinitrophenylhydrazine (DNPH) was purchased from Ajax Finechem (Taren Point, NSW, Australia). Trichloroacetic acid (TCA) and guanidine hydrochloride were purchased from Merck (Darmstadt, Germany). OxiSelect™ N^{ε}-(carboxymethyl) lysine (N^{ε}-CML) ELISA kit was purchased from Cell Biolabs (San Diego, CA, USA). Cyanidin-3-rutinoside chloride (C3R; Fig. 1) was synthesized from quercetin-3-rutinoside according to a previous method [25].

2.2. Glycation of bovine serum albumin (BSA)

The glycated BSA formation was prepared according to the previous method [26]. 0.5 mL of BSA (10 mg/mL) was incubated with 0.46 mL of monosaccharides (0.1 M ribose, 0.5 M fructose, 0.5 M glucose or 0.5 M galactose) and 0.04 mL of C3R (0.125–1.00 mM) or AG (1.00 mM) as positive control in 0.1 M phosphate buffer saline (PBS), pH 7.4 at 37 °C for 2 weeks. C3R was dissolved in dimethylsulfoxide (DMSO) and added to the solution to give a final concentration of 4% DMSO. The solution containing BSA in 4% DMSO was used as a blank of control in the experiment.

2.3. Determination of AGE formation

The glycated BSA formation was quantified by a spectrofluorometer at excitation and emission wavelengths 355 and 460 nm. The percentage of inhibition was calculated by following equation below:

$$\text{Inhibition of fluorescent AGEs}(\%) = [((F_C - F_{CB}) - (F_S - F_{SB}))/(F_C - F_{CB})] \times 100$$

Where F_C and F_{CB} were the fluorescent intensity of control with monosaccharides and blank of control without monosaccharides, F_S and F_{SB} were the fluorescent intensity of sample with monosaccharides and blank of sample without monosaccharides.

2.4. Determination of N^{ε}-(carboxymethyl) lysine, (N^{ε}-CML)

N^{ε}-CML, a major non-fluorescent AGE structure, was determined by using enzyme linked immunosorbant assay (ELISA) kit according to the manufacturer's manual. The concentration of N^{ε}-CML was calculated from the standard curve of N^{ε}-CML-BSA.

2.5. Determination of fructosamine

The level of fructosamine was analyzed by nitrobluetetrazolium (NBT) assay [27]. The glycated BSA (10 μL) was incubated with 0.5 mM NBT in 0.1 M carbonate buffer (90 μL), pH 10.3 at 37 °C. The absorbance was measured at 590 nm. The concentration of fructosamine was calculated by using the different absorption at 10 and 15 min time points compared with the standard 1-deoxy-1-morpholino-fructose (1-DMF) curve.

2.6. Determination of β-amyloid cross structure

The level of β-amyloid cross structure was measured according to a previous method with minor modifications [28]. Briefly, the glycated BSA (50 μL) was incubated with 64 μM thioflavin T (50 μL) in 0.1 M PBS for 60 min at 25 °C. The fluorescence intensity was measured at excitation 435 nm and emission 485 nm.

2.7. Determination of protein carbonyl content

Protein carbonyl content is a marker of oxidative protein damage which was analyzed according to a previous method with slight modifications [29]. Briefly, 0.1 mL of glycated BSA was incubated with 0.4 mL of 10 mM 2,4 dinitrophenylhydrazine (DNPH) in 2.5 M HCl at 25 °C in dark. After 60 min, protein were precipitated by 0.5 mL of 20% (w/v) trichloroacetic acid (TCA) for 5 min on ice, and then centrifuged at 10,000 g for 10 min at 4 °C. The protein pellet was washed two times with 1 mL of 1:1 (v/v) ethanol/ethyl acetate mixture. Finally, the protein pellet was dissolved in 0.25 mL of 6 M guanidine hydrochloride. The absorbance was measured at 370 nm. The carbonyl content of each sample was calculated based on the