ANTIGLYCATION PROPERTIES OF RESVERATROL AND GLUCOSAMINE

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ABSTRACT

The non-enzymatic glycosylation (glycation) is a natural process occurring in living organisms, which is associated with aging and diabetic complications. This non-enzymatic reaction affects not only long-lived proteins, such as eye crystalline, but also DNA, RNA and aminolipids. The long-standing search for substances that can inhibit this process is still on-going. In this study we examined the effect of two natural compounds, resveratrol and glucosamine, on the formation of early and fluorescent advanced glycation end products (AGEs). We found that resveratrol inhibited formation of fluorescent AGEs in bovine serum albumin (BSA) glycated with D-ribose, with inhibition percentage of 80 %, while glucosamine inhibited the formation of the same products in the same test system only by 4 % compared to a control sample of glycated BSA. Both test compounds, resveratrol and glucosamine, did not inhibit the formation of early glycation products in the glycated BSA. Our results showed that resveratrol has strong antiglycation activity in contrast to glucosamine demonstrating very low antiglycation potential.

Keywords: resveratrol, glucosamine, non-enzymatic glycosylation.

INTRODUCTION

Resveratrol (4-hydroxystyryl) benzene-1,3-diol) is a natural compound found in grapes, red wines, berries, tea and other plant products [1 - 5] (Fig. 1). Plants have evolved to synthesize this compound in response to bacterial or fungi infections [6]. Resveratrol was first isolated by the Japanese scientist Takaoka in 1939 when he studied the plant white hellebore (Veratrum album) [7]. Resveratrol exists in both cis and trans isomeric forms but of these two, trans-resveratrol is the more stable and biologically more active isomer. This trans isomer has three hydroxyl groups that interact with different macromolecules and participate in neutralization of free radicals in different cells [8, 9].

The study of the biological activity of resveratrol began with the discovery that moderate consumption of wine has beneficial effects on health. It has been found that wine polyphenols, such as resveratrol had anti-inflammatory, cardio protective and antioxidant activities. Later, a number of studies showed that resveratrol may also have antitumor activity and antidiabetic effects [10 - 15].
Glucosamine is a natural monosaccharide (Fig. 2), which is part of chitosan, connective tissues and gastrointestinal mucosal membranes. This natural product is used for prevention and treatment of osteoarthritis, and induced insulin resistance [16, 17]. Another study showed that glucosamine inhibits the glucose transporter system, especially the protein GLUT-4, thereby decreasing glucose levels in adipose tissue and skeletal and cardiac muscles. There is also evidence that glucosamine participates in proteoglycan synthesis and in biosynthesis of biopolymers of joints and bones [18 - 20].

Non-enzymatic glycosylation (glycation) is a process occurring in all living organisms, from bacteria to humans. The process occurs spontaneously between reducing sugars and biomolecules such as proteins, DNA, RNA and lipids containing a free amino group. As a result, early and advanced glycation end products are formed, which are covalently linked to the different biopolymers. The accumulation of glycation products in cells and tissues disrupts their biological functions and is one of the causes of complications related to diabetes mellitus, aging and different neurodegenerative diseases [21 - 24].

In this work we tested resveratrol and glucosamine for activity as inhibitors of glycation. We chose glucosamine because its free amino group could interact with the carbonyl group of reducing sugars. In this way, by blocking carbonyl groups, it could potentially reduce the glycation capacity of any reducing sugar. In case of resveratrol, we chose this compound in our experiments because it has antioxidant properties. From the research of Baynes et al. it became clear that in living organisms, Advanced glycation end products (AGEs) are generated by a combination of oxidation and glycation reactions [25]. Therefore, we speculated that suppression of oxidation by resveratrol may potentially result in suppression of the glycation process.

**EXPERIMENTAL**

**Materials and chemicals**

*Trans*-resveratrol, glucosamine, aminoguanidine, L-lysine, hydrochloric acid and Tris were obtained from Sigma-Aldrich. Bovine serum albumin, sodium chloride and D-ribose were obtained from Gibco Chemical Co. All reagents were of analytical grade. Milli-Q ultrapure water was used in all analyses.

**Glycation of bovine serum albumin (BSA) with D-ribose**

BSA at a concentration of 2 mg mL⁻¹ was incubated with 200 mM or 40 mM D-ribose for 12 or 15 days at 37°C. In the different experiments, 20 mM resveratrol, 20 mM aminoguanidine, 20 mM glucosamine or 20 mM L-lysine were added to the incubation mixture. At the end of the incubation, samples were dialyzed against TS buffer (0.14 M NaCl, 0.01 M Tris HCl, pH 7.5) for 24 hours and centrifuged for 30 min at 12 000 g.

**Nitroblue tetrazolium (NBT) reduction assay**

Early glycation products in glycated BSA were determined by a modified nitroblue tetrazolium (NBT) reduction assay. BSA samples (100 μl) were mixed with 1 ml 100 mM sodium carbonate buffer (pH 10.8) containing 0.25 mM NBT and incubated at 37°C for 30 min. Absorbance at 525 nm was measured against distilled water and the content of early glycation products was determined using an extinction coefficient of 12 640 cm⁻¹ M⁻¹ for monoformazan (a product formed by the reduction of NBT by early glycation adducts). Data are presented as means ± SD (n = 3).

**Fluorescence spectroscopy**

Fluorescent AGEs in the glycated BSA were measured on a Shimadzu spectrofluorometer RF-5000U by recording the emission at 440 nm upon excitation at 370 nm as described elsewhere [26]. Relative fluorescence units (RFU) were recorded and the percentage of AGEs formation inhibition was calculated as follows: % of inhibition = [1 - (RFU sample/RFU Positive control)] x 100. Each experiment was performed in triplicate and a 6 % measurement error for this method was included, as determined in preliminary studies.
RESULTS AND DISCUSSION

In our experiments, resveratrol and glucosamine at 20 mM concentrations were incubated with BSA (2 mg mL\(^{-1}\)) and 200 mM D-ribose for 12 days at 37°C. The concentration of glycation products was measured by two different methods: a) NBT reduction assay (to measure the early glycation products, i.e. ketoamines) and fluorescence spectroscopy (to quantify the fluorescent AGEs in glycated BSA).

We found that resveratrol and glucosamine increased the quantity of early glycation products in BSA glycated with D-ribose. We measured 40 % rise in the concentration of early glycation products when glycation of BSA was performed in the presence of resveratrol or glucosamine as compared to the positive control of BSA glycated with D-ribose (Fig. 3).

In order to detect the formation of advanced glycation end products in the glycated BSA, AGEs-specific fluorescence was recorded. The levels of fluorescent AGEs in BSA after 12 and 15 days of incubation with 200 mM D-ribose and 20 mM resveratrol, 20 mM glucosamine or 20 mM aminoguanidine are shown in Fig. 4(A) and Fig. 4(B).

Resveratrol demonstrated strong inhibitory activity reducing the AGEs level to 79 % (day 12) and 80 % (day 15) of the control BSA glycated with D-ribose. Glucosamine had marginal impact on BSA glycation. This compound reduced the formation of fluorescent glycation products only by 4 % as compared to the control. However, from Fig. 4 it can be seen that resveratrol has a weaker antiglycation activity as compared to one of the most effective glycation inhibitors aminoguanidine which inhibited glycation by 95 % (12 days) and 96 % (15 days). To confirm the anti-glycation potential of resveratrol, we also conducted an experiment in which we glycated BSA with D-ribose in the presence of L-lysine. In our previous research we found that L-lysine has a strong proglycating effect which was also confirmed in this experiment. When lysine is added to D-ribose and BSA, the amount of fluorescent glycation products increased almost twofold compared to control of glycated BSA after 12 days of incubation at 37°C (Fig. 5).

When resveratrol was added to the same reaction mixture containing BSA, D-ribose, and L-lysine,

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Fig. 3. The concentration of early glycation products in BSA (2 mg mL\(^{-1}\)) glycated for 12 days with 200 mM D-ribose (1) in the presence of 20 mM resveratrol (2) or 20 mM glucosamine (3).

Fig. 4. Fluorescent AGEs in 2 mg mL\(^{-1}\) BSA (1) incubated with 200 mM D-ribose (2) for 12 (A) or 15 (B) days in the presence of 20 mM resveratrol (3), 20 mM glucosamine (4) and 20 mM aminoguanidine (5).
it was observed 18.4 % reduction of the formation of fluorescent glycation adducts as compared to the control. The strong anti-glycation effect of resveratrol was confirmed by the 71 % reduction in the concentration of fluorescent AGEs in BSA when the glycation reaction was performed in the presence of resveratrol only (Fig. 5). All these results clearly showed that resveratrol is a strong inhibitor of the non-enzymatic glycosylation process.

The question of the exact mechanism of acting of this substance neutralizes the formation of glycation products remains open. From the results presented above it becomes clear that resveratrol does not inhibit the formation of early glycation products, but reduces the accumulation of fluorescent AGEs in the glycated BSA. How does this happen? Yim et al. have shown that incubation of methylglyoxal with L-alanine results in the formation of three free radical species: the cross-linked methylglyoxal dialkylimine radical cation, the enediol radical anion of methylglyoxal and the superoxide radical anion as determined by EPR spectroscopy [27]. Such radicals have the potential to enhance glycoxidation reactions. It appears that at the beginning of the glycation process highly reactive radicals are formed which accelerate the formation of AGEs in later glycation stages. Neutralization of these radicals by radical scavengers such as resveratrol results in inhibition of AGEs formation as we observed. Consistent with this hypothesis, in our study resveratrol inhibited only in the formation of fluorescent AGEs in the glycated BSA, but not that of early glycation products. Note that the formation of early glycation products is oxidation-independent.

CONCLUSIONS

The investigated compounds resveratrol and glucosamine demonstrated divergent effects on the formation of fluorescent AGEs in BSA glycated with D-ribose in vitro. Resveratrol inhibited the formation of fluorescent AGEs with an inhibition percentage of 80 %, while glucosamine inhibited the formation of the same products only by 4 %. Both test compounds, resveratrol and glucosamine, did not inhibit the formation of early glycation products. The strong antglycation effect of resveratrol on AGEs formation is attributable to its ability to scavenge free radicals formed during the intermediate and advanced stages of the glycation reaction.

Fig. 5. Fluorescent AGEs in 2 mg mL⁻¹ BSA (1) incubated with 40 mM D-ribose (2) for 12 days in the presence of 20 mM lysine (3), 20 mM lysine and 20 mM resveratrol (4) and 20 mM resveratrol (5).

REFERENCES

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