SHORT COMMUNICATION

Antiglycation activity of β-glucogallin from Asparagus racemosus

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ABSTRACT
The increased formation and accumulation of advanced glycation end products (AGEs) has been implicated in pathogenesis of various chronic ailments, including diabetes-associated secondary complications, atherosclerosis, aging, inflammatory and neurodegenerative diseases. Therefore, inhibition of AGEs formation is an imperative strategy for alleviating diverse pathologies. Here, we have demonstrated the AGEs inhibitory activity of β-glucogallin, isolated for the first time from the roots of Asparagus racemosus. β-glucogallin significantly mitigated fructose-, glucose- and methylglyoxal-induced glycation of bovine serum albumin (BSA). Also, the presence of β-glucogallin decreased fructosamine and protein carbonyls content, and increased thiol group content in the fructose-BSA system. These activities of β-glucogallin from Asparagus racemosus underscore its likely pharmacological potential for impeding AGEs-related metabolic disorders.

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1. Introduction
Glycation is a non-enzymatic, impulsive chemical reaction between the free reducing sugars and amino groups of proteins, DNA, or lipids, resulting in the formation of highly reactive advanced glycation end products (AGEs) (Kim et al. 2017). The rate of glycation is directly proportional to the concentration of reducing sugar, time and temperature (Singh et al. 2001). Therefore, persistent hyperglycemia during
unmanaged diabetes accelerates the rate of glycation reaction, leading to increased formation and accumulation of AGEs (Negre-Salvayre et al. 2009). The glycation reaction, also known as Maillard reaction, is a complex cascade of reactions, usually classified into three phases: initiation, propagation and advance phase (Deo et al. 2013). The initiation phase of glycation comprises reaction of reducing sugars such as glucose, fructose, or ribose with free amino group of proteins, nucleic acids or lipids to form unstable Schiff bases. Further progression of the reaction includes structural rearrangement of these Schiff bases to form relatively more stable and irreversible Amadori products (Jahan and Choudhary 2015). The propagation phase comprises non-oxidative rearrangement and hydrolysis of Amadori products, marked by the formation and accumulation of highly reactive intermediates, $\alpha$-dicarbonyl compounds including, 3-deoxygluconosone, glyoxal and methylglyoxal (Thorlalley 1996). During the final advanced phase of glycation, these highly reactive $\alpha$-dicarbonyl compounds react with arginine and lysine residues of the proteins to produce heterogeneous irreversible adducts, the AGEs.

The AGEs are involved in the pathogenesis of various ailments, including diabetes-associated secondary complications, atherosclerosis, Alzheimer’s disease, aging, inflammatory, and neurodegenerative disorders (Ott et al. 2014). The implication of glycation process in the pathogenesis of diverse metabolic pathologies warrants discovery and development of efficient antiglycating agents for the effective management of these pathologies (Lushington and Barnes 2019). Several classes of antiglycating agents with diverse modes of action have been identified from both natural products and synthetic molecules; but could not be developed further for therapeutic uses because of their low effectiveness, unfavourable side effects or poor pharmacokinetic properties (Jahan and Choudhary 2015). Therefore, the discovery and development of novel antiglycating agents for the management of diverse pathologies has recently attracted attention.

*Asparagus racemosus* Willd. or Shatavari, belonging to the family *Asparagaceae*, is a well-known medicinal plant in Indian subcontinent (Singh 2016). It has been extensively utilized in Ayurveda to benefit overall human health (Amjad et al. 2020). It is bestowed with several pharmacological properties like anticancer (Biswas et al. 2018; Sharma and Jaitak 2020), antioxidant (Ghosh and Mitra 2020), anti-inflammatory, anti-diabetic and hypolipidemic (Wesam et al. 2018; Ahsan et al. 2019). Phytochemical investigation of this plant has demonstrated the presence of asparagusic acid (Jansen 1948; Mitchell and Waring 2014) and other sulphur-containing constituents (Waring et al. 1987; Venditti and Bianco 2020), that have reported for the various bioactivities (Venditti et al. 2013; Salemme et al. 2016). Highly acclaimed as a galactagogu’ for lactating mothers, this plant is a household name, containing steroidal saponins and related compounds as major phytococonstituents in the roots (Kashyap et al. 2020; Tantapakul et al. 2020). In the present study, phytochemical investigation of the roots of this plant led us to isolate and identify $\beta$-glucogallin with antiglycation potential. As per previous reports, $\beta$-glucogallin has been isolated for the first time from *Asparagus racemosus* in a quantitative yield. It was evaluated at the different steps of glycation process and found to exhibit significant antiglycation activity, suggesting its potential
2. Results and discussion

Glycation of biomolecules is a deleterious process involved in onset of various pathologies (Ahmed 2005). Therefore, discovery of potent antiglycating molecules is a promising approach for the management of diverse diseases. Here, we undertook phytochemical investigation of the roots of A. racemosus leading to the isolation of β-glucogallin, and further evaluated it for antiglycation activity. β-glucogallin was characterized with the help of NMR (1H & 13C) and mass spectrometry data (Supplementary data, Figures S1–S3), which was in good coherence with the reported literature values (Puppala et al. 2012).

The glycation process is generally slow at physiological conditions and takes several days to weeks to complete. Therefore, to test antiglycation activity, we incubated β-glucogallin with fructose or glucose for 15 or 21 days at 370C in dark. Incubation of BSA with fructose resulted in a time-dependent increase in fluorescence intensity at day 15 and day 21, indicating the formation of fructosylated-BSA. The presence of aminoguanidine (5 mM), a prototype antiglycation molecule caused around 86.0% decrease in fluorescence intensity of fructosylated-BSA (P < 0.0001; Figure 1B) at day 15 and day 21, suggesting the inhibition of fructose-induced fluorescent-AGEs formation. Likewise, presence of β-glucogallin prevented fructose-induced fluorescent-AGEs formation in a concentration-dependent manner, with significant decrease observed at a minimal concentration of 75 μM (P < 0.001) at day 15 and day 21 (Figure 1B and C). Incubation with 300 μM of β-glucogallin caused around 67.0% inhibition of glycation reaction at day 15 and day 21 (P < 0.0001). Next, we assessed the effect of β-glucogallin on glucose-induced AGEs formation. Similar to fructose, incubation of BSA with glucose facilitated the formation of glucosylated-BSA, characterised by a profound increase in fluorescence intensity at day 15 (Figure 1D). However, the fluorescence intensity of glucosylated-BSA was lesser than that of fructosylated-BSA at the same concentration of both the sugars, verifying that fructose is more active in Maillard reaction than glucose (Suarez et al. 1989). Consistent to the effect on fructosylated-BSA formation, presence of aminoguanidine or β-glucogallin significantly hampered the formation of glucose-induced fluorescent-AGEs (glucosylated-BSA) in a concentration-dependent fashion (Figure 1D) with around 65.5% inhibition at 300 μM concentration after 15 days (P < 0.0001). These findings suggested antiglycation activity of β-glucogallin to prevent fructose- or glucose-induced AGEs formation.

Given that glycation is a multistep process; inhibition of glycation may involve numerous mechanisms that can hamper or prevent the formation of AGEs. Some of these mechanisms including, scavenging of free radicals, decreasing the formation of reactive carbonyl species, blocking carbonyl and dicarbonyl moieties of the reducing sugars, Schiff bases and Amadori products to inhibit the progression of Maillard reaction, metal ion chelation, and breaking the covalent cross-links in pre-formed AGEs, may reduce the AGEs accumulation (Jahan and Choudhary 2015). We tested antiglycation potential of β-glucogallin at different steps of glycation process. The initiation
Figure 1. Antiglycation activity of β-glucogallin (A) isolated from the roots of A. racemosus. β-glucogallin (β-GG, at indicated concentration in μM) or aminoguanidine (AG, 5 mM) was incubated with BSA in presence of fructose (Fru) for 15 days (B) and 21 days (C), or with glucose (Glu) for 15 days (D) or with methylglyoxal (MG) for 48 h (G) and formation of fluorescent AGEs was measured, as described in experimental section. Amount of fructosamine (E), protein carbonyls (F) and thiols (H) was measured in fructose-BSA system after 15 days. Results are mean ± SEM (n = 4). β-glucogallin was dissolved in water, hence compared with BSA + Fru condition. Aminoguanidine was dissolved in DMSO, hence compared with BSA + Fru with DMSO condition (BSA + Fru + DMSO). **P < 0.01, ****P < 0.0001.
phase of glycation is characterised by the formation of unstable Schiff bases that undergoes chemical rearrangements to generate more stable Amadori products such as fructosamine. We tested the effect of \( \beta \)-glucogallin on fructosamine formation. Incubation of BSA with fructose for 15 days caused a significant increase in fructosamine level, compared to BSA alone. Presence of aminoguanidine (5 mM) or \( \beta \)-glucogallin in a concentration-dependent manner inhibited fructosamine formation during fructosylation of BSA (Figure 1E). Presence of 300 \( \mu \)M of \( \beta \)-glucogallin caused around 34.0% decrease in fructosamine level (\( P < 0.0001 \)). Further progression of glycation process involves intermediary formation of highly reactive dicarbonyl compounds. Incubation of BSA with fructose significantly enhanced the content of dicarbonyls after 15 days (\( P < 0.0001 \)), which was significantly inhibited in presence of aminoguanidine (23.0%, \( P < 0.01 \)) or \( \beta \)-glucogallin at 150 and 300 \( \mu \)M concentrations (53.0% and 63.0% respectively, \( P < 0.0001 \), Figure 1F). Enhanced generation of the triose in intermediary nutrient metabolism can facilitate the formation of dicarbonyls like methylglyoxal by a non-oxidative mean. For example, distinct metabolism of fructose favours formation of triose during glycolysis and contributes to methylglyoxal accumulation (Gugliucci 2017). Therefore, obstruction of these dicarbonyl moieties can impede the glycation process. We assessed the effect of \( \beta \)-glucogallin on methylglyoxal-mediated AGEs formation. Incubation of BSA with methylglyoxal (30 mM) for 48 h significantly enhanced the fluorescence intensity (\( P < 0.0001 \)), indicating formation of glycated-BSA. Presence of aminoguanidine or \( \beta \)-glucogallin significantly restricted the methylglyoxal-mediated AGEs formation (Figure 1G). Altogether, these findings suggested the potential role of \( \beta \)-glucogallin to inhibit fluorescent AGEs formation by multiple mechanisms at the initiation and propagation phases of glycation process.

Furthermore, breaking of the covalent cross-links in pre-formed AGEs may reduce the AGEs accumulation, and associated derangements (Jahan and Choudhary 2015). The activity of \( \beta \)-glucogallin to break pre-formed AGEs was assessed by incubating with fructosylated-BSA. The solution of fructosylated-BSA was dialysed to remove any free fructose to halt further AGEs formation, and then incubated with \( \beta \)-glucogallin for 7 days. Presence of \( \beta \)-glucogallin had no significant effect on fluorescence intensity of fructosylated-BSA, suggesting that \( \beta \)-glucogallin had no significant effect on preformed AGES, but it impeded AGES formation at multiple steps of glycation process. Glycation of proteins is escorted by oxidation at certain amino acids that may alter structure and functions of the proteins (Rai et al. 2021). We assessed protein oxidation during glycation by measuring content of thiol groups in fructosylated-BSA. Incubation of BSA with fructose for 15 days led to around 30% (\( P < 0.0001 \)) decrease in thiol groups compared to BSA alone. Presence of aminoguanidine or \( \beta \)-glucogallin in a dose-dependent manner rescued glycation-mediated decrease in thiol group content of BSA (Figure 1H). Altogether, our findings suggested the protective role of \( \beta \)-glucogallin from the \textit{A. racemosus} in prevention of protein glycation through multiple mechanisms.

Protein glycation and accumulation of AGEs have been associated with pathogenesis of various ailments. Glycation impairs protein functions by changing their structural properties and stability (Fournet et al. 2018). Moreover, the interaction of AGEs with receptors for AGEs (RAGE) activates multiple intracellular signalling leading to the
modulation of gene expression and activation of pro-inflammatory responses, which contribute to metabolic complications related to diabetes such as nephropathy, retinopathy, neuropathy, and atherosclerosis (Takeuchi et al. 2010). Given the data for anti-glycation activity, β-glucogallin from the *A. racemosus* may be pursued further for the development and designing of new molecules as a therapeutic agent for diabetes- or other AGEs-related metabolic complications.

3. Experimental
All experimental procedures are described in the *supplementary material* section.

4. Conclusion
β-glucogallin from the *A. racemosus* displayed potent antiglycation activity mediated through its action on different phases of glycation process. These findings suggested the likely potential of β-glucogallin for the development and designing of new analogues of β-glucogallin as therapeutic agent for AGEs-mediated metabolic complications.

Disclosure statement
No potential conflict of interest was reported by the authors.

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