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Vanillin restrains non-enzymatic glycation and aggregation of albumin by chemical chaperone like function

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ABSTRACT

Vanillin a major component of vanilla bean extract is commonly used a natural flavoring agent. Glycation is known to induce aggregation and fibrillation of globular proteins such as albumin, hemoglobin. Here we report the inhibitory potential of vanillin toward early and advanced glycation modification and amyloid like aggregation of albumin based on the determination of both early and advanced glycation and conformational changes in albumin using circular dichroism. Inhibition of aggregation and fibrillation of albumin was determined based on amyloid specific dyes i.e., Congo red and Thioflavin T and microscopic imaging. It was evident that vanillin restrains glycation of albumin and exhibits protective effect toward its native conformation.

1. Introduction

Glycation induced structural and functional changes in proteins have been identified as a major link between diabetes and its severe complications such as retinopathy, neuropathy and cardiovascular complications [1,2]. Glycation involves the reaction of reducing sugars or their metabolic intermediates with the free amino groups in amino acid side chains. Advanced glycation end products (AGEs) are the end products of glycation reaction, which are characterized due to their high reactivity, cross linking, and fluorescence properties [3]. Under hyperglycemic conditions where serum proteins are directly exposed to the glycemic fluctuations, such reaction can occur at an increased rate leading to the accumulation of glycated protein aggregates [4].

Albumin is a major transport protein in blood and has been extensively studied for its structure and functions [5–9]. The glycated form of albumin has been proposed to be a better marker for the diagnosis of diabetes patients when compared to the $HbA1_c$ [10,11]. Mass spectroscopic studies in recent have been successfully used in order to determine the glycation sites in albumin [12]. Glycation modulates structure and function of albumin leading to the development of crippled drug carrier with impaired carrier function [13]. Due to the complexity of the reaction different approaches have been proposed to inhibit the glycation and formation of advanced glycation end products such as; (a) carbonyl scavenging (b) inhibition of early glycation reaction (c) inhibition of advanced glycation reaction (d) masking of lysine residues in proteins and (e) protecting the native protein conformation. Even numerous studies in the recent have reported, the antiglycation activity of both synthetic and natural compounds [14–16] but yet none of them are being clinically used at present. Considering the pathological consequences of non-enzymatic reaction in the development of complications in diabetes and its effect on protein structure function, the focus of current research is toward identifying potent glycation inhibitors. Despite strong antiglycation activity, the synthetic compound such as aminoguanidine, has been removed from the clinical trial due to severe side effects [17] With this motivation and considering the present scenario, we focused toward the identification of natural compounds with potent antiglycation activity [18–20].

Vanillin is a major component of vanilla bean extract, which is extensively used as a natural food flavoring agent. The antioxidant, antidepressant, and anti-proliferative activities of vanillin have been reported based on both *in vitro* and *in vivo* studies [21]. Also, *in vivo* studies using mice have proven the clinical efficacy of vanillin [22]. In this study, we attempted to demonstrate the antiglycation activity of vanillin, which also prevented the amyloid like aggregation of albumin. The inhibition of glycation by vanillin was determined based on the estimation of early and advanced glycation end products and quantification of lysine modification. Circular dichroism analysis was used in order to determine the effect of vanillin on glycation induced secondary structural changes in albumin. The inhibitory effects of vanillin toward

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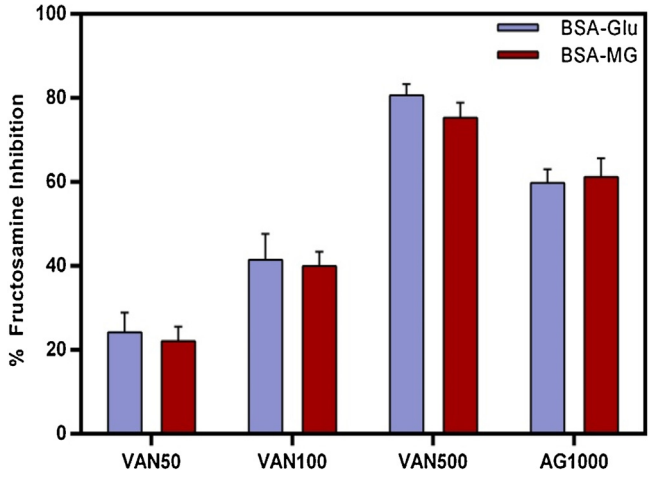

Fig. 1. Effect of vanillin on early glycation of albumin in presence of methylglyoxal (MG) and glucose (Glu). Results are means ± standard deviations of three different assays.

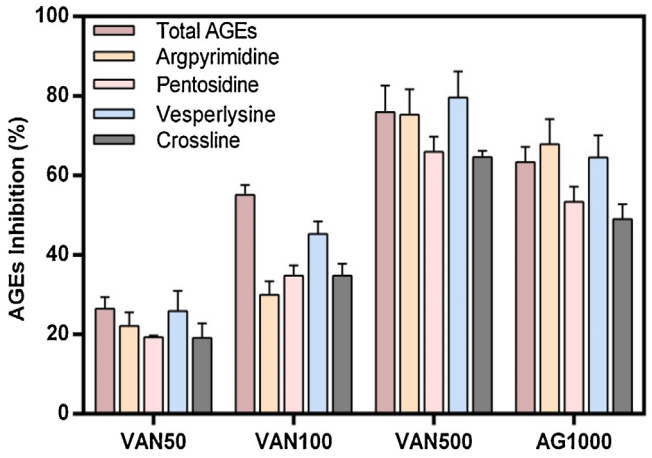

Fig. 2. Effect of vanillin on advanced glycation end products (AGEs). Results are means ± standard deviations of three different assays.

glycation induced fibrillation was determined based on Thioflavin T (ThT), and Congo red (CR) binding and confocal imaging.

2. Materials and methods

2.1. *Chemicals and* in vitro *glycation of BSA*

Bovine serum albumin (BSA), vanillin (VAN), methylglyoxal (MG), aminoguanidine (AG), 2,4,6-trinitrobenzenesulfonic acid (TNBSA), amyloid specific dyes i.e., Congo red (CR) and Thioflavin T (ThT) were purchased from Sigma, USA. Nitro blue tetrazolium (NBT) was obtained from SRL, India. All other chemicals of AR grade for the preparation of buffers were purchased from Merck, India. *In vitro* glycation was carried out by incubating BSA (10 mg ml^{-1}) in dark with glucose (25 mM) and MG (10 mM) for the period of 30 days. A pre-incubation of 10 min was carried out with the test compound before the addition of glucose/MG. The control samples include BSA alone, BSA + Glucose and BSA + MG. AG was used as a positive control.

2.2. *Determination of fructosamine adduct*

Fructosamine assay as described by Baker et al. [23] was used with slight modifications. Briefly, glycated protein samples were

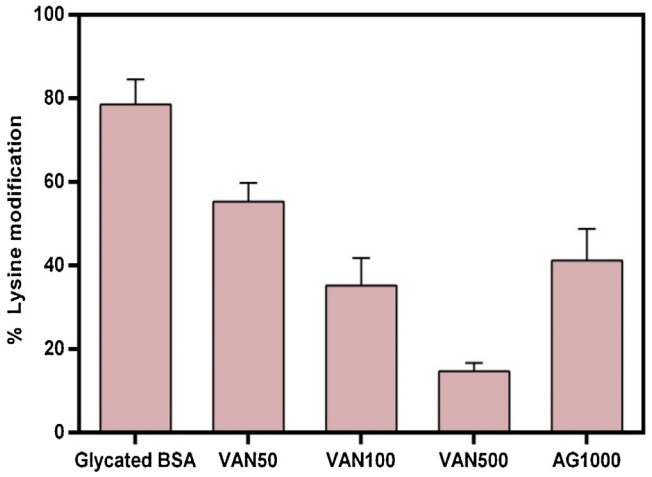

Fig. 3. Effect of vanillin on lysine modification as determined by TNBSA assay. Results are means ± standard deviations of three different assays.

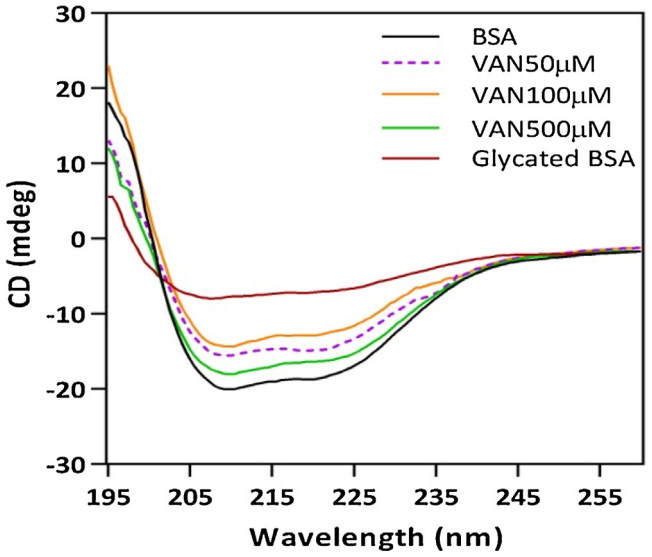

Fig. 4. Effect on vanillin on the conformation of BSA. Far-UV CD spectra of native BSA, glycated control and in the presence of vanillin (50, 100 and 500 μM).

incubated with NBT (150 μM) for 30 min. NBT was prepared using sodium carbonate buffer (100 mM). The absorbance was recorded at 530 nm using Thermo Scientific Evolution-201 spectrophotometer.

2.3. *Inhibition of fluorescent AGEs*

AGEs fluorescence was determined using Jasco-FP8200 spectrofluorimeter. The excitation (λ_{ex}) and emission (λ_{em}) wavelengths for different AGEs have been given in detail in our earlier work [14]. The results were expressed in the form of percent inhibition.

2.4. *Quantification of lysine modification*

Quantification of lysine modification was carried out by TNBSA assay [24]. Briefly, glycated protein (1 mg ml^{-1}) sample was incubated with TNBSA (0.01%) for 2 h at room temperature. Following incubation 10% SDS and 1 N HCl was added and absorbance was read at 335 nm using Thermo Scientific Evolution-201