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Destructive effect of non-enzymatic glycation on catalase and remediation via curcumin

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ABSTRACT

Non-enzymatic glycation of proteins is a post-translational modification that is produced by a covalent binding between reducing sugars and amino groups of lysine and arginine residues. In this paper the effect of pathological conditions, derived from hyperglycemia on bovine liver catalase (BLC) as a model protein was considered by measuring enzyme activity, reactive oxygen species (ROS) generation, and changes in catalase conformational properties. We observed that in the presence of glucose, the catalase activity gradually decreased. ROS generation was also involved in the glycation process. Thus, decreased BLC activity was partly considered as a result of ROS generation through glycation. However, in the presence of curcumin the amount of ROS was reduced resulting in increased activity of the glycated catalase. The effect of high glucose level and the potential inhibitory effect of curcumin on aggregation and structural changes of catalase were also investigated. Molecular dynamic simulations also showed that interaction of catalase with curcumin resulted in changes in accessible surface area (ASA) and pKa, two effective parameters of glycation, in potential glycation lysine residues. Thus, the decrease in ASA and increase in pKa of important lysine residues were considered as predominant factors in decreased glycation of BLC by curcumin.

1. Introduction

For a long time, the cause of diabetic complications was an open question but today there is consensus that these complications are associated with chronic hyperglycemia. Thus, high blood sugar is recognized as a causative factor in the pathogenesis of diabetes complications [1]. Exploration of the non-enzymatic glycation theory and its relation with excessive plasma and tissue glucose reinforced that these can exert pathological effects [2]. The glycation reaction typically takes place between free ε-amino groups of some amino acid residues especially lysine, arginine and sometimes histidine of a protein and glucose. This reaction leads to formation of a Schiff base, subsequently chemical rearrangement and more complex products (Amadori products). This process eventually results in the formation of advanced glycation end products (AGE) as an important glycation toxin. Numerous studies have demonstrated that glycation can cause gradual deterioration in the structure and function of proteins [3–5]. Oxidative stress is also a widely accepted consequence of chronic hyperglycemia [6,7], which is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms present in biological systems [8,9]. Recent studies indicated that if this ROS is not rapidly removed, it may also affect proteins through either glycoxidation pathways [10] or lipoproxidative production of reactive aldehydes [11]. There is also a causal relationship between hyperglycemia-induced ROS generation and intracellular AGE production [12]. In addition, there are numerous studies on effective antioxidant compounds which can help lower the markers

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indicative of oxidative stress in diabetic subjects and animals [13,14].

Curcumin as a yellow natural phenol from *Curcuma longa* is a principal component of turmeric, and is commonly used as a food flavoring and food coloring agent. Among the various antioxidants, many studies have been devoted to curcumin antioxidant and anti-inflammatory properties in the past few decades. Moreover, many studies have demonstrated that curcumin has potential beneficial effects against diabetes complications, and it can reduce hyperlipidemia [15], ameliorate renal lesions [16], modulates α-synuclein toxicity, positive effects on Parkinson's disease [17], and delay the development of cataract [18]. Also it has been shown that curcumin can reduce protein glycosylation and oxygen radical generation in human red blood cells [19], and blood glucose levels in type 2 diabetic KK-Ay mice [20]. However, how curcumin can delay or inhibit complications of diabetes remains elusive.

Antioxidant enzymes have also an important role in the defense system for negating destructive effects of ROS *in vivo*. Thus, every factor that may undermine the activities of antioxidant enzymes can lead to accumulation of ROS and subsequent oxidative damage to proteins. Glycation can induce structural changes in enzymes starting from conformational alterations, aggregation, formation of disulphide bonds, production of covalent cross-links, and inactivation of enzymes [21]. In the antioxidant defense system, catalase is the only antioxidant enzyme which is markedly glycated in old rats [22]. Catalase is one of the important enzymes in elimination of ROS, particularly hydrogen peroxide. It coverts hydrogen peroxide into less reactive gaseous oxygen and water molecule. Catalase has a predominant role in reducing the severity of complications associated with diseases that are impacted by products of ROS generation including diabetes. Glycation of catalase can lead to a decrease in activity and loss of its antigenicity [23]. Catalase activity in blood of patients with diabetes [24,25], and similarly in patients with schizophrenia and atherosclerosis [26], is also significantly lower than healthy human subjects.

The aim of present study was to determine how hyperglycemia may affect catalase, and if curcumin can play a remediation effect. For this purpose, we first determined the effect of glycation on functional and conformational of catalase experimentally, and evaluated the adverse effects of glycation on these parameters. We next examined whether curcumin plays a protective role in these interactions.

2. Materials and methods

2.1. Materials

BLC (Bovine Liver Catalase) and curcumin were purchased from Sigma–Aldrich. The β-D-glucose was purchased from Fluka. Hydrogen peroxide (H_2O_2, 30% solution standardized by a UV–vis spectrometer at 240 nm), Luminol (5-amino-2,3-dihydro-1,4-phthalazine) were from Merck. Gold (III) chloride hydrate (HAuCl4) was from Sigma. Other chemicals were of reagent grade and all the solutions were prepared using distilled water.

2.2. Sample preparation

The concentration of stock solution of catalase was measured by its absorbance at 405 nm, using $3.24 \times 10^5\ M^{-1}\ cm^{-1}$ for the molar extinction coefficient [27] and 250,000 Da for the molecular mass of BLC. BLC (0.175 mg/ml) was incubated with 16 mM of β-D-glucose and with 700 nM of curcumin solution, separately and in combination. Two sample series were prepared. In the first series, to measure probable produced ROS, which can consume as substrate by catalase, sodium azide solution 0.02% was used to inhibit catalase. The second series were used to measure activity and other structural changes, catalase was not inhibited by sodium azide. Curcumin stock solution (9 mM) was prepared in methanol. This stock solution was further diluted with double distilled water to reach 700 nM in final concentration and diminished methanol effects. The ratio of concentration of catalase to curcumin in all samples was 1:1. More concentration of curcumin could not be used due to solubility limitation in phosphate buffer. The incubation of catalase with reagents was performed under physiological (37 °C and dark) and sterile conditions for7 days.

2.3. Methods

2.3.1. Measurement of reactive oxygen species (ROS)

Regarding the possible production of H_2O_2 and its consummation as a substrate by BLC, sodium azide solution (0.02%) was used to inhibit BLC. To ensure that BLC is completely inhibited, activity of the solution was measured by UV–Vis spectrophotometry, and no catalase activity was detected (not shown). To determine possible ROS production under experimental conditions, chemiluminescence spectroscopy was applied using a chemiluminescence spectrophotometer (Synergy H4 Hybrid Reader; BioTek) as previously described by Hu [28]. Briefly, 5 μL $HAuCl_4$ solution (dissolved in distilled water) and 5 μL of luminol solution (dissolved in100 mM sodium carbonate pH 11) were injected into the samples in the first step. Chemiluminescence emission was gathered after mixing. Every measurement was done at least three times.

2.3.2. Catalase activity and kinetic measurements

Catalase activity was measured using a UV–Vis spectroscopy (Varian UV–Vis spectrophotometer, model Carry 100 Bio). To determine catalase activity before and after the addition of sugar, the instrument was set at 240 nm and hydrogen peroxide was used as the catalase substrate. Changes in the rate of hydrogen peroxide decomposition come from changes in catalase activity. All samples were incubated up to 7 days at 37 °C, and enzyme activity was measured in triplicate on days: 0, 1, 3, 5 and 7. Control values represent the catalytic activity of catalase incubated without glucose and curcumin at the same times.

2.3.3. Circular dichroism spectroscopy

Far- UV CD spectra of samples were acquired using a circular dichroism spectropolarimeter model JASCO J-810. The wavelength range was 200–260 nm with a band width of 1 nm, and scan speed of 20 nm/min with spectral resolution of 1 nm was used. All CD spectra were corrected by subtracting of phosphate buffer spectrum from all CD spectra. Secondary structural elements of samples were analyzed using a CDNN program.[1]

2.3.4. Thermal aggregation measurements

Thermal aggregation of BLC was measured using a UV–Vis spectrophotometry (Varian UV–Vis spectrophotometer, model Carry 100 Bio). To assess the effects of glycation and antioxidant on thermal aggregation of BLC, the instrument was set at 360 nm and it was followed at 55 °C for 30 min.

2.3.5. NR-fluorescence spectroscopy

To detect solvent-exposed hydrophobic surface of protein, ANS (1-anilinonaphthalene-8-sulfonic acid) is usually used as a fluorescence dye. However, the range of excitation of curcumin falls in emission of ANS/bis-ANS, and energy transfer between ANS/bis-

[1] http://bioinformatik.biochemtech.uni-halle.de/cdnn.