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Studies to reveal the nature of interactions between catalase and curcumin using computational methods and optical techniques

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ABSTRACT

Curcumin is an important antioxidant compound, and is widely reported as an effective component for reducing complications of many diseases. However, the detailed mechanisms of its activity remain poorly understood. We found that curcumin can significantly increase catalase activity of BLC (bovine liver catalase). The mechanism of curcumin action was investigated using a computational method. We suggested that curcumin may activate BLC by modifying the bottleneck of its narrow channel. The molecular dynamic simulation data showed that placing curcumin on the structure of enzyme can increase the size of the bottleneck in the narrow channel of BLC, and readily allow the access of substrate to the active site. Because of the increase of the distance between amino acids of the bottleneck in the presence of curcumin, the entrance space of substrate increased from 250 $Å^3$ to 440 $Å^3$.

In addition, the increase in emission of intrinsic fluorescence of BLC in presence of curcumin demonstrated changes in tertiary structure of catalase, and possibility of less quenching. We also used circular dichroism (CD) spectropolarimetry to determine how curcumin may alter the enzyme secondary structure. Catalase spectra in the presence of various concentrations of curcumin showed an increase in the amount of α-helix content.

1. Introduction

Increased levels of reactive oxygen species (ROS), as a result of diminished capacity of intracellular anti-oxidant defense systems, contribute to pathogenesis of many diseases including cancer, diabetes, and cardiovascular diseases. ROS are chemically reactive molecules which are mainly byproducts of normal cell metabolism. The mass generation of ROS may lead to cell damage and likely affect proteins, lipids, and nucleic acids. As an important oxidant agent, hydrogen peroxide is one of ROS species whose increased production can damage the β-cells in the pancreatic Langerhans islands [1–3]. Hydrogen peroxide has also been reported as an inhibitor of insulin signaling [4]. Catalase is one of the antioxidant enzymes playing a predominant role in elimination of ROS, particularly hydrogen peroxide, by converting it into a less reactive gaseous oxygen and water molecules [5]. A catalase deficiency may increase the likelihood of developing type-2 diabetes mellitus. Catalase activity in blood of patients with diabetes was significantly lower than healthy human subjects [6,7], and similarly in patients with schizophrenia and atherosclerosis [8]. Acatalasemia (<10% of normal activity) and hypocatalasemia (~50% of normal activity) are two categories of genetic deficiencies of erythrocyte catalase [9].

Many studies have investigated different components and various factors that affect catalase activity, and subsequently result in improvement of the enzymatic efficiency through modifications of some amino acid residues [10–13]. Based on the X-ray crystal structure of bovine liver catalase (BLC), there is a main channel in the protein structure that is responsible for connecting the deeply buried heme with the enzyme surface. The steric hindrance and the hydrophobic nature of this channel restrict substrate access [14]. The length of the main channel, from the protein surface to the

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heme, is 22–55 Å [15]. The first 20 Å of the main channel, starting from the surface, is not as significant as the final 15 Å, since this region of the protein does not restrict the accessibility of substrate to the heme cavity.

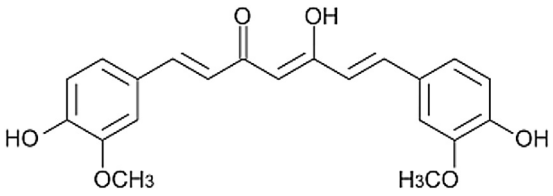
The value of 0–15 Å above the heme, starting from conserved Asp^{127}, is a narrow channel and plays a significant role in the catalytic function of catalase [16]. Chelikani et al. suggested that the three-dimensional organization and shape of the main channel regulate the efficiency of catalase activity [15]. Fourteen amino acid residues of the narrow channel are as follow: Val^{73}, His^{74}, Val^{115}, Asp^{127}, Pro^{128}, Asn^{147}, Phe^{152}, Phe^{153}, Phe^{160}, Phe^{163}, Ile^{164}, Gln^{167}, Trp^{185}, and Leu^{198}. The conserved amino acid residues of the narrow channel can significantly affect enzyme activity [17]. Zamocky et al. demonstrated that the side chains of these residues are important in construction of the channel's bottleneck. They showed substitution of Val^{111}, 8 Å away from the heme group, with a smaller amino acid residue such as Ala increased the access of large substrates and enhanced enzyme peroxidatic activity [18]. Chelikani et al. pointed out the importance of a potential field between Asp^{181} with a negative charge, and heme with a positive charge. They reported that the mutation of Asp ^{181}, at a distance of 12 Å from the heme, to Ala, Asn, Gln, Ile, or Ser reduced enzymatic activity [19]. Also, it is well accepted that the amino acid side chains forming the narrow channel play a significant role in substrate access and catalase reaction. It was reported that catalase from *Exiguobacterium oxidotolerans* T-2-2T (EKTA catalase) can decompose hydrogen peroxide more rapidly than BLC or *Micrococcus luteus* catalase (MLC). This finding has been attributed to the larger bottleneck of the EKTA narrow channel [17].

A significant antioxidant effect has been reported for curcumin (diferuloylmethane), the most effective component of turmeric plant. This important natural compound is also used as a flavor in Indian cuisine [20–23], and it is responsible for the yellowish color of curry. Many experimental studies show that curcumin is used as a remedy in human illnesses including cancer, diabetes, Alzheimer, hepatic disorders, rheumatism, anorexia and Parkinson [24–27]. It is suggested that curcumin has a significant ability to inhibit ROS production, which is caused by high glucose levels in erythrocyte of diabetic patients [28]. Curcumin can also bind specifically to α-synuclein oligomers and reduce its toxicity in Parkinson's disease [29]. The molecular mechanism by which curcumin prevents or attenuates complication of various diseases is not yet known. Thus, studying the effect of curcumin, as an important antioxidant, on the structural and functional properties of proteins and enzymes that are involved in diseases has not only theoretical significance but also clinical applications. In the current study, we demonstrated that interaction of curcumin with catalase resulted in activation of the enzyme. We have studied the mechanism of this activation of BLC by curcumin employing various computational and experimental techniques. We demonstrated that this interaction has important impact on the conformation, and possibly accessibility of the enzyme active site.

2. Materials and methods

2.1. Material

Curcumin and bovine liver catalase (BLC) were purchased from Sigma–Aldrich. The concentration of stock solution of catalase was measured by its optical density at 405 nm, using $3.24 \times 10^5\ M^{-1}\ cm^{-1}$ for the molar extinction coefficient [30] and 250,000 Da for the molecular mass of BLC. Curcumin stock solution (9 mM) was prepared in methanol. This stock solution was further diluted with twice distilled water and working solution was prepared freshly before using. The used concentration of methanol did not affect the structure and activity of catalase because it was diluted about 1000 times. All the tests were carried out at room temperature and at least 3 times.



Scheme 1. Curcumin formula.

2.2. Methods

2.2.1. Enzyme assay

Catalase activity was determined by measuring the rate of hydrogen peroxide decomposition. The samples containing catalase (30 nM) with different concentrations of curcumin were prepared in phosphate buffer (pH 7.4) 1 h before testing. The reaction was recorded immediately after mixing 10 μL, per samples, with 990 μL hydrogen peroxide (12 mM). The reduction of absorbance at 240 nm resulted from H_2O_2 elimination was followed. One unit of activity was defined as the amount of enzyme that decomposes 1 μM hydrogen peroxide in 1 min. The concentration of stock solution of catalase was determined by measuring its absorbance at 405 nm, using $3.24 \times 10^5\ M^{-1}\ cm^{-1}$ for the molar extinction coefficient [30] and 250,000 Da for the molecular mass of BLC. The concentration of hydrogen peroxide was obtained by UV–vis spectrophotometry (Varian UV–vis spectrophotometer, model Carry 100 Bio) at 240 nm using $43.6\ M^{-1}\ cm^{-1}$ as an extinction coefficient (Scheme 1).

2.2.2. Computational methods

Avogadro program was used to draw the structure of curcumin [31]. Gaussian 09 (Rev. D.01) was used for the optimization of curcumin structure [32]. The B3LYP method using the 6–31G(d) basis set was applied in the mentioned step, and the minimum nature of the structure was confirmed by the absence of the imaginary frequency [32]. AutoDock Vina was used to perform the docking studies and the result of this step was analyzed using AutoDock Tools 4 [33,34]. The point numbers equal to 100 in the directions X and Y, and 110 in the direction Z were used to prepare the grid box to cover the whole protein structure. Iterated Local Search global optimizer implanted in the AutoDock Vina was used in docking study [35]. MD simulations were carried out using Amber12 suite of program [36]. The general Amber force field (GAFF) parameters were applied for the curcumin using antechamber program of the Amber Tools 12 [37,38]. The RESP charges were calculated for the heme moiety at the B3LYP/6–31 g (d) level of theory. The complex of the final result of docking studies was used as the starting point of the MD simulation. The system was solvated using TIP3P water model [39]. At first 2000 steps of minimizations were performed to remove the close contacts. The first 1000 steps carried out using steepest descent algorithm and the second 1000 steps using conjugate gradient algorithm. The Langevin thermostat was used to adjust the temperature to 300 K with gradual increase during 25,000 steps, and then kept at this temperature for 75,000 steps [40]. Two successive runs were carried out, including 100,000 steps for each one to obtain the constant density and equilibrate the system. Finally, a 10 ns simulation was carried out.

The 1TGU.pdb was used to obtain the structure of the protein. All the water molecules and ligand, except the heme moieties, were removed from the pdb file. Because the attachment positions for the curcumin on catalase have not been previously determined,