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Protective effects of aspirin on the function of bovine liver catalase: A spectroscopy and molecular docking study

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ABSTRACT

Amongst all different kinds of analgesic drugs, aspirin (acetylsalicylic acid) is most commonly used to relieve minor pains, reduce fever and to prevent inflammation. To explore the possible side effects of aspirin on the antioxidant defense system of the liver, we analyzed the conformational and functional changes of bovine liver catalase (BLC) in the presence of aspirin, using different spectroscopic techniques including UV–Visible, fluorescence and circular dichroism (CD). No major changes in activity were seen for BLC in the presence of aspirin as observed by kinetics studies. Our results showed that the intrinsic fluorescence of BLC is quenched by aspirin via a static mechanism. Also thermodynamic parameters were calculated showing that van der Waals forces and hydrogen bonds play a major role in this interaction. Moreover, the UV–Vis absorption data in agreement with Trp fluorescence results demonstrated that the microenvironment of Trp residues in BLC is disrupted by aspirin binding. CD results have further shown a significant change in the secondary structure of BLC with an increase in the α-helical content from 24.2% in native protein to 47.4% in BLC-aspirin and a decrease in β-pleated sheets content from 21% in native BLC to 12% upon aspirin addition. The molecular docking results are in well agreement with the experimental data confirming that there exists one binding site for aspirin on BLC at the distance of Forster theory. Additionally, the docking calculations showed that a complex was formed between aspirin and BLC consistent with the data on the intrinsic fluorescence intensity of BLC based on the static quenching mechanism. Finally, all the results from this study have indicated that the addition of aspirin leads to the unfolding of the three-dimensional structure of BLC and can be considered as the side effect of this drug on the antioxidant system of the liver. It might be suggested that in the presence of high concentrations of H_2O_2, aspirin can protect BLC activity through a mechanism in which the inactive form of the enzyme (compound II) is reduced to the active form.

1. Introduction

Catalase or hydrogen peroxide oxidoreductase (EC: 1.11.1.6) belongs to the large group of heme-containing proteins [1]. This unique enzyme exists in peroxisomes of aerobic cells, which catalyzes the dismutation of hydrogen peroxide (H_2O_2) to H_2O and O_2, hence protects them against reactive intermediates of oxygen generated through aerobic metabolism [2,3]. Detection of inherited catalase deficiency called Acatalasemia by Takahara in 1946, showed the importance of catalase activity [4]. Acatalasemia (an autosomal recessive trait) was thought to be a relatively harmless congenital abnormality in the beginning. However, accumulation of H_2O_2, as one of the factors which is capable of causing oxidative damage to macromolecules, due to acatalasemia is associated with several human diseases including: diabetes mellitus, Alzheimer's disease, vitiligo and tumors. Considering these connections, researchers have suggested that acatalasemia should not be marked as a normal enzyme deficiency [5–7].

Bovine liver catalase (BLC) is a member of heme-containing monofunctional catalases also characterized as typical catalases [8]. Structural studies using X-ray crystallography has shown that BLC contains a homo-tetrameric structure (PDB ID: 1TGU). Each subunit of this symmetric tetramer is composed of 506 residues, with a molecular weight of ~60 kDa and a tightly-bound NADPH ($K_D \leq 5$ nM), as a cofactor [9–11]. NADPH, which is a common constituent of mammalian catalases, is not required for catalytic activity of BLC. Previous studies have provided evidences that NADPH preserves BLC from suicide inactivation by H_2O_2. During the catalytic reaction and contiguous production of H_2O_2, an inactive species of the enzyme BLC (compound II) is produced. NADPH acts as an electron donor through elimination or inversion of compound II to produce the active form of the catalase (ferri-catalase), preventing the reaction from termination [12–15]. The active site of BLC contains

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four heme groups (protoporphyrin IX) that are thoroughly buried in the core of the tetramer. The prosthetic heme group is approachable by three channels, only for specific substrates which are appropriate in size to insert into these channels [10–16].

Aspirin (acetylsalicylic acid) as a well-known therapeutic agent was first used for its anti-inflammatory effect, a characteristic that situates it into a large group of medications called nonsteroidal anti-inflammatory drugs (NSAIDs). The anti-inflammatory activity of aspirin is achieved through inhibiting the cyclo-oxygenase (by acetylating serine 529 in cyclo-oxygenase-1 isoform) and consequently impairing the prostaglandin synthesis pathway [17,18]. For a long time aspirin has been considered as an effective drug in the secondary prevention of cardiovascular disease especially in patients, ischaemic strokes and cancer prevention due to its antioxidant activity [19].

Previous studies have shown that aspirin interacts with different proteins including albumin, transferrin, immunoglobulin G and A through the mechanism of acetylation. Furthermore, the inhibitory effect of aspirin on tyrosinase activity has been reported. Aspirin-mediated inhibition of tyrosinase is both reversible and dose-dependent [20,21].

Considering the fact that catalase is largely localized in the liver as the major organ in which consumed drugs are metabolized and detoxified, the manifestation of toxic side effects of drugs is detected faster in the liver compared to other organs. Therefore, analyzing the alterations in liver catalase activity and structure can be an appropriate parameter in evaluating the effects of different kinds of drugs on the body. In this research, we attempt to focus on the impacts of aspirin, as a highly used medicine, on the function and structure of bovine liver catalase.

2. Materials and methods

2.1. Materials

Aqueous suspension of bovine liver catalase (BLC, C30-500MG) was purchased from Sigma (34 mg/ml). Hydrogen peroxide (H_2O_2) 30% was purchased from Merck. Aspirin (M.W. 180.157 g/mol) was synthesized in the Faculty of Pharmacy, Tehran University of Medical Sciences. Disodium hydrogen phosphate (MW 141.96 g/mol) and sodium dihydrogen phosphate (MW 137.99 g/mol) used in the buffer preparation were purchased from Merck. The concentration of H_2O_2 stock solution was determined spectrophotometrically through measurement of its maximum absorbance at 240 nm and using the extinction coefficient of 40 M^{-1} cm^{-1} for H_2O_2 [22].

2.2. Methods

2.2.1. Kinetics studies of the native BLC

The activity of BLC was assayed by following the decrease in H_2O_2 absorbance at 240 nm (A_{240}) due to its decomposition by BLC using the UV–Visible spectrophotometer (GBC Cintra UV–Visible 101 spectrophotometer) [23]. The activity measurements was carried out in a 3 ml cuvette containing sodium phosphate buffer (50 mM, pH 7.0) and appropriate concentrations of H_2O_2 (10–90 mM). The enzymatic reactions were initiated after adding 10 μl of diluted BLC suspension (0.56×10^{-3} mg/ml) to the mixtures. All experiments were performed three times and changes at A_{240} were monitored every 1 s for 1 min at 25 °C.

2.2.2. Kinetics studies of BLC in the presence of aspirin

In order to investigate the possible effects of aspirin on the function and activity of BLC, H_2O_2 (70 mM) in sodium phosphate buffer (50 mM, pH 7.0) was incubated in the absence and presence of different concentrations of aspirin (0, 5, 10, 13, 25, 33, 46, 53, 63 and 83 μM) for 3 min. Subsequently, BLC (0.56×10^{-3} mg/ml) was added to the incubated solutions and the difference in absorbance was recorded at 25 °C.

2.2.3. Fluorescence spectroscopy studies

With the intention of evaluating the three- dimensional structural changes of BLC with various concentrations of aspirin, the intrinsic fluorescence studies of BLC was carried out using a Cary Spectrofluorimeter at two temperatures of 25 °C and 37 °C. For this purpose, the fluorescence emission spectra of native BLC and BLC in the absence and presence of different concentrations of aspirin (2, 4, 6, 8, 10, 12, 14, 16, 20, 24 and 28 μM) were recorded with the excitation wavelength (λ_{ex}) set at 290 nm and the emission wavelength (λ_{em}) ranged between 300 and 500 nm. The experimental solution containing 0.2 mg/ml BLC in 50 mM sodium phosphate buffer, pH 7.0, was incubated with each concentration of aspirin for 3 min prior to measurements.

2.2.4. Circular dichroism (CD) spectroscopy

The far-UV CD spectra (190–260 nm) of 0.1 mg/ml of the native and modified BLC in 50 mM sodium phosphate (pH 7.0) were collected by a circular dichroism spectrometer model 215 (Aviv instruments Inc.), using a cell of 1 mm path length at different temperatures of 25 °C and 37 °C. In addition, the alterations in the percentage of secondary structure elements of BLC in the absence and presence of various concentrations of aspirin (8, 16 and 32 μM) were estimated by the CDNN program and the data expressed as molar ellipticity (deg cm^2 $dmol^{-1}$).

2.2.5. UV–Vis absorption spectra

All UV–Visible absorption spectra of BLC (0.23 mg/ml) influenced by increasing amounts of aspirin (0, 5, 10, 13, 25, 33, 46, 53, 63 and 83 μM) were taken by a GBC Cintra UV–Visible 101 Spectrophotometer in a wavelength scanning mode. The spectral range of 200–700 nm was defined.

2.2.6. Molecular docking study

The molecular docking study using Autodock 4.2 software was done to demonstrate the mode of interaction between aspirin and BLC [24]. The BLC crystal structure (PDB Id: 1TGU) was obtained from the RCSB Protein Data Bank [25]. In this work we carried out theoretical evaluations only on chain A of BLC as all four chains of BLC were similar. Input files for docking study were prepared based on previous works [26]. In addition, the Lamarckian genetic algorithm was selected for binding site searching on the whole chain A of BLC [27]. The molecular structure of aspirin at theoretical level of AM1 method using Hyperchem8.0.6 program was modeled and optimized to minimal energy [28]. The molecular docking input files and visualization of the docking results as well as the theoretical analysis of the binding site and mode of interaction between aspirin and BLC was performed using AutoDock Tools 1.5.4 package and VMD package [29,30].

3. Results

3.1. Kinetics studies

3.1.1. Analysis of BLC activity

In this research, the UV–Vis spectrophotometric method was used to explore the catalytic activity of BLC in the absence and presence of aspirin. In this approach, the rate of substrate breakdown (H_2O_2) was measured at 240 nm and the reduction in H_2O_2 absorption against unit time was considered as BLC activity. A fixed concentration of 0.56×10^{-3} mg/ml of BLC in the presence of additional concentrations of H_2O_2 in the range of 10–90 mM (from 0.33 to 2.5 K_m) was used to calculate the BLC function at 25 °C. In agreement with previous reports, we found that with increasing the H_2O_2 concentration from 10 to 70 mM, a marked increase in enzyme activity was observed, whereas at higher concentrations of H_2O_2 (more than 70 mM), a clear reduction in activity was observed [23,31]. Reduction in the activity at higher concentrations of H_2O_2 as shown in the Michaelis–Menten plot (Fig. 1a) elucidates the suicide inactivation process [32]. In order to determine the accurate kinetic