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Generation of reactive oxygen species via inhibition of liver catalase by oxalli-palladium: A spectroscopic and docking study

Azam Gholamian[a], Adeleh Divsalar[a,*], Maryam Saiedifar[b], Behafarid Ghalandari[c], Ali Akbar Saboury[d], Bahare Koohshekan[a]

[a] Department of Cell & Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[b] Material and Energy Research Center, Tehran, Iran
[c] Department of Medical Nanotechnology, Applied Biophotonics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran
[d] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran

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ABSTRACT

In the present study, the side effects of an anti-cancer compound of oxali-palladium on the function and structure of bovine liver catalase (BLC) were investigated using multiple spectroscopic (fluorescence, circular dichroism (CD) and UV–vis) and molecular docking methods. Results of kinetics study showed a noncompetitive inhibition of the enzyme for oxali-palladium. Addition of various concentrations of oxali-palladium caused a gradual reduction in the intrinsic fluorescence emission intensity of BLC due to quenching the fluorescence of BLC via a static type of quenching mechanism. Also addition of oxali-palladium to the enzyme led to increasing content of α-helix and decreasing of β-pleated sheet and random coil structures. The molecular docking study in well coherent with fluorescence spectroscopy illustrated that there is one binding site for oxali-palladium on bovine liver catalase. Docking results confirmed that static quenching is occurred whereas oxali-palladium is located at the distance of Föster theory. Moreover, docking examination in agreement with binding analyzing revealed electrostatic interaction is dominant driving force in oxali-palladium binding to BLC. According above results, it can be concluded that inhibition of BLC by anticancer drug of oxali-palladium increases the content of reactive oxygen species (ROS) which is one of the mechanisms of different anticancer drugs.

1. Introduction

Hydrogen peroxide (H_2O_2) is one of the reactive oxygen species that produced from mitochondrial electron transport and β-oxidation of fatty acids [1,2]. It has toxic effects on the cells. Therefore, its value should be adjusted in cells. Two paths namely exist for remove toxic effects of H_2O_2 including: glutathione peroxidase and catalase [3]. Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6; CAT) is an antioxidant enzyme that found almost in all livings organisms. Catalase is known as one of the most common enzymes in plant and animals. In human organs, it has highest activity in liver, kidney and erythrocytes. It catalyzes the decomposition of hydrogen peroxide to oxygen and water at a two-step reaction. At first step, one hydrogen peroxide converts to water and ferri-catalase changed to compound I (porphyrin$^+$-Fe^{4+} = O). At the second step, compound I oxides the second hydrogen peroxide to oxygen molecule and ferri-catalase and water released. So catalase protects cells from the toxic effects of hydrogen peroxide [4,5].

Bovine liver catalase (BLC) (MW = 240000 Da) belongs to the heme- containing enzymes. This enzyme is a tetrameric enzyme with four equal subunits, which has 506 amino acids. Each subunit of the enzyme consists of a heme prosthetic group that is buried at depth of enzyme [5]. Catalase have hydrophobic channel that named access channel because it converts substrate to active site [6]. BLC, like human catalase, can strongly bind to NADPH as cofactor (one molecule for each subunit). The roles of NADPH is protect catalase from inactivation by H_2O_2. Indeed NADPH is known to counterbalance the capacity of hydrogen peroxide to convert catalase to an inactive state (compound II) [5].

Hydrogen peroxide has functional roles in cell adhesion, motility, cell proliferation and metastasis, so catalase by detoxification of hydrogen peroxide can change these functions [7]. Previous reports have shown that catalase was extensively considered during of cancer because the decreasing of catalase stabilizes as particular sign of tumor tissues. Also, level of mRNA and protein of catalase reduced in cancer cells. Moreover, decreasing in catalase activity is a risk fac-

* Corresponding author.
E-mail address: divsalar@khu.ac.ir (A. Divsalar).

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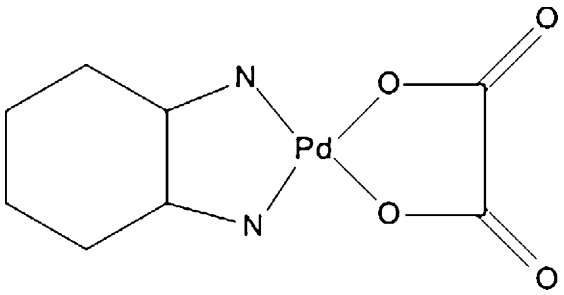

Fig. 1. The molecular structure of oxali-palladium.

tor for cancer [8]. So, investigation on changes in catalase activity in the presence of anticancer drugs can help to better understanding the treatment of various cancers.

Metal complexes have applied in the treatment of several diseases including cancers, bacterial infections, anti-inflammatory, diabetes, and other disorders. Among metal complexes, platinum complexes have used in cancer treatment mainly [9]. The first platinum drugs that used for cancer treatment was *cis*-platin. Various side effects of *cis*-platin such as: renal toxicity, neurotoxicity and ototoxicity and drug resistance, resulted to appearance of second and third generation of platinum drugs including carboplatin and oxali-platin [10,11]. Resistance to platinum complexes and also their side effects limited its clinical application then, promising scientists to design new palladium complexes. Palladium complexes, as the other group of metal complexes with antineoplastic potential, have similar activity as *cis*-platin but have shown lower side effects on renal toxicity [12,13]. Palladium complexes as well as platinum complexes can react with the amine groups of DNA, RNA and proteins [12–14]. The mechanism of action from palladium complexes is reaction with amino groups of DNA, RNA and proteins that produced palladium-DNA adducts which related with antitumor activity. Oxali-palladium (Fig. 1) is one of the of palladium complexes with anticancer activity [13–15].

Liver is a main tissue in body that involves in the catabolism and detoxification of various drugs and it is a target of toxic and carcinogenic effects of many drugs like anticancer drugs [16], so the structure and function of catalase which has high activity in liver, can change by drugs. Also, previous researches indicated that catalase has important roles in cancer and response to anticancer drugs, then detection and determination of liver catalase activity is a useful parameter in estimating of the influences of various anticancer drugs such as doxorubicin, paclitaxel and docetaxel [17]. Therefore, taking a chemical substance such as drugs can affect the catalytic activity of catalase either increasing it or inactivating it. Then, in the present study, the side effects of an anticancer compound of oxali-palladium on the function and structure of liver catalase were investigated using different spectroscopic (UV–vis, fluorescence and circular dichroism (CD)) and docking techniques.

2. Materials and methods

Catalase (bovine liver catalase) was purchased from Sigma Aldrich. Oxali-palladium was synthesized in our laboratory according to previous reports [18]. H_2O_2 was purchased from Merck Company and the concentration of stock solution of H_2O_2 was determined by measurement of its molar extinction coefficient ($3.92 \times 10^4\ M^{-1}\ cm^{-1}$) at 240 nm [19].

2.1. Kinetic studies of BLC in the presence of oxalli-palladium

The catalase activity was determined according to previous methods of Beers and Sizer [16]. BLC catalyzes the decomposition of hydrogen peroxide into water and oxygen. The assays were carried out in 1000 μl cells and the BLC activity was determined at 25 °C by determination the rate of hydrogen peroxide decomposition at 240 nm in sodium phosphate (50 mM, pH 7) buffer, during 60 s by carry UV–vis spectroscopy. Substrate concentration in all assays was 0.3–2.5 K_m [5].

In order to determination of drug effects on the enzyme activity, the enzyme (21 nM) activity was investigated in the presence of various concentrations of oxali-palladium (0–13 μM) and fixed concentration of substrate (70 μM) at 25 °C.

2.2. Determination of inhibition mechanism

In order to determination of the inhibition mechanism of BLC by oxali-palladium, the enzyme (2.3 nM) activity in the presence of fixed and different concentrations of oxali-palladium (2, 4 and 8 μM) was measured at various concentrations of substrate. Then, according to Lineweaver–Burk and secondary plots, the inhibitory mechanism of oxali-palladium on catalase was investigated [18,19].

2.3. Fluorescence measurements

Intrinsic fluorescence spectra of catalase (0.83 nM) was carried out using Varian Spectrofluorimeter, Cary Eclipse model with 1 cm quartz cells, and the excitation wavelengths fitted at 290 nm at different temperatures of 25 and 37 °C. Then, the fluorescence spectra were recorded at 300–500 nm. The tertiary structural changes of the enzyme were studied in the absence and presence of various concentrations of oxali-palladium (0–800 μM) in 50 mM phosphate buffer, pH 7 at different temperatures of 25 and 37 °C.

2.4. Circular dichroism measurements

CD spectral measurements in the far-UV regions (190–260 nm) were performed on an Aviv Spectropolarimeter Model-215 in a 1 mm cell at two temperatures of 25 and 37 °C. According to statistical methods that implemented in CD software of CDNN, changes in the secondary structure of catalase (41 nM) in the absence and presence of various concentrations of oxali-palladium (0, 180, 360 and 720 μM) was determined in 50 mM phosphate buffer, pH 7 at different temperatures of 25 and 37 °C.

2.5. UV–vis measurements

Changes in UV–vis spectra of catalase (0.17 mg/ml) upon interaction with various concentrations of oxali-palladium (0, 2, 4, 16 and 24 μM) were recorded using a Carry spectrophotometer.

2.6. Molecular docking study

Molecular docking examination was performed in order to mode of interaction demonstration between BLC and oxali-palladium using Autodock 4.2 software [20]. The crystallography structure of BLC was obtained from the RCSB Protein Data Bank based on 1TGU [21] PDB Id. The BLC is made of four similar subunits. Then, theoretical evaluation was carried only on chain A of BLC. According to previous studies the input files were prepared so that for binding parameters calculations such as binding site searching on whole chain A of BLC the Lamarckian genetic algorithm was used [22,23]. The molecular structure of oxali-palladium was modeled using HyperChem 8.0.6 program [24]. Oxali-palladium geometry was optimized by minimizing the energy using quantum mechanics (QM) method that implemented in Gaussian 98 program [25] at theoretical level of B3LYP with 6-31G (d, p) basis set for C, N, O and H atoms and LanL2DZ basis set for Pd atom. In addition, to