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How a promising anti-cancer derivative of palladium consisting phen-imidazole ligand affects bovine liver catalase functionality

Roohollah Ghobadi[a], Adeleh Divsalar[b,*], Ali Reza Harifi-Mood[a,*], Ali Akbar Saboury[c], Mahboubeh Eslami-Moghadam[d]

[a] Department of Physical Chemistry, Faculty of Chemistry, Kharazmi University, Tehran, Iran
[b] Department of Cell & Molecular Sciences, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[c] Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
[d] Chemistry & Chemical Engineering Research Center of Iran, Tehran, Iran

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ABSTRACT

Promising background of using Nobel metal complexes such as cisplatin in cancer therapy has been emerged as encourage for scientists to design new species with the outlook of finding acceptable alternatives holding less side effects. That's why study of interactions of promising complexes with special targets such as enzymes seems necessary. As similarity between coordination chemistry of Pd(II) and Pt(II) has been an excuse for scientists to find an alternative to cisplatin, we investigated the biological effects of $[Pd(dach)(FIP)](NO_3)_2$ compound containing a novel phen-imidazole ligand, FIP, and diamino cyclohexane (dach) on bovine liver Catalase (BLC) structure and function. Due to UV–vis study, with an increase in palladium compound's concentration to 30 μM, the enzymatic activity reduced gradually. Afterwards in higher concentrations remained constant at around 65%. Meanwhile K_m, k_{cat} and v_{max} followed an upward trend which confirm mixed inhibitory mechanism.

Although based on fluorescence quenching measurements in 25 and 37 °C catalase activity changes slightly, three-dimensional environment surrounding chromophores of the enzyme structure changes considerably in the presence of palladium complex. According thermodynamic parameters of this interaction, it is believed that hydrophobic interactions are influential in the binding process. Furthermore, CD spectroscopy data revealed that Pd(II) complex makes the secondary structure of BLC less stable. Despite remarkable structural changes of BLC due to hydrophobic interactions with ligands, its function was not influenced considerably. The results might expand horizons of medicinal chemists who are looking for metallic compounds as anticancer drugs with fewer side effects.

1. Introduction

In the recent years, metal complexes with medical or diagnostic characteristics have gained more attentions [1–4]. Obviously structural modification of aforesaid drugs can change their influence and biological activity. That's why rational design and synthesis of novel anticancer drugs, holding variety of ligands and metals, has considerable importance for interpretation and identification of their mechanism of actions [5,6].

Cisplatin has been emerged as the most efficient and popular metal complex in the treatment of various cancerous malignancies, which was accidently discovered by Rosenberg and co-workers [7,8]. Nowadays, this complex is as one of the best-selling anti-cancer drugs worldwide. Owing to some drawbacks like restricted application to a limited spectrum of tumors, some evidences of toxicity and inducing resistance against DNA replication [1,9,10], seeking appropriate alternatives appear to be crucial. Palladium due to comparable coordination chemistry with platinum and some advantages like higher solubility of its complexes compared to Pt, has attracted attentions as a potential candidate [4,11–16].

It has been reported that Pd(II) complexes have cytotoxicity activity against head and neck squamous cancer, prostate cancer, ovarian cancer, glioma, human colorectal adenocarcinoma, malignant melanoma, osteogenic sarcoma, human chronic myelogenous leukemia, breast cancer, lung cancer and human cervical epithelial cancer [1].

Significant rate of ligand exchange and consequent rapid hydrolysis is one of the major drawbacks of palladium complexes which is addressed by using bulky chelating ligands [1].

Abbreviations: BLC, bovine liver catalase; CD, circular dichroism; FIP, 2-(furan-2-yl)-1H-pmidazo[4,5-f] [1,10]phenanthroline; dach, 1,2-diaminocyclohexane

* Corresponding authors.
E-mail addresses: divsalar@khu.ac.ir (A. Divsalar), harifi@khu.ac.ir (A.R. Harifi-Mood).

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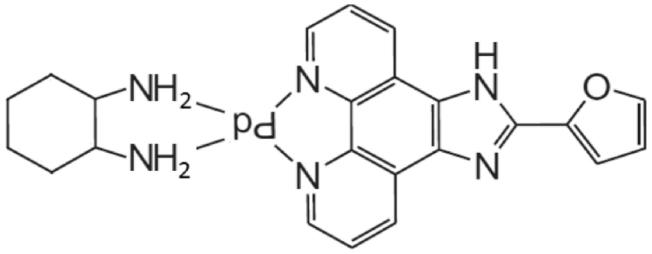

Fig. 1. The molecular structure of 2-(Furan-2-yl)-1H-Imidazo[4,5-f][1,10]1,2 diaminocyclohexane Palladium nitrate ([Pd(dach)(FIP)]$(NO_3)_2$).

Meanwhile, Phenanthroline, imidazole and diaminocyclohexane are well-known ligands due to special characteristics. Structural similarity between imidazole and histidine, the most abundant amino acid, is a driving force in binding process of imidazole to macromolecules like proteins [4,17,18]. Moreover, polypyridines such as phenanthrolines can easily interact with DNA via intercalation mechanism. That's why they are widely used in the synthesis of metal complexes for therapeutic uses [4,19–22]. The newly synthesized ligand of [Pd(dach)(FIP)]$(NO_3)_2$ (Fig. 1) which FIP: 2-(Furan-2-yl)-1H-Imidazo[4,5-f][1,10] Phenanthroline, which is the combination of imidazole and Phenanthroline, was bound to palladium [4].

To investigate probable side effects of this metallic compound, bovine liver catalase (BLC) was selected as a target molecule.

Catalase (H_2O_2: H_2O_2 oxidoreductase EC 1.11.1.6) is a highly active, ubiquitous enzyme which occurs in almost all aerobically respiring organisms and serves in a part to protect the cells from the toxic effects of hydrogen peroxide [23]. Moreover, as catalase regulates the polarization of adipose tissue macrophages, it's deficiency increase probability of insulin resistance [24]. Disorders like acatalasemia (often the result of mutation in the CAT gene) may be a risk factor for age related diseases such as diabetes, neoplasms and atherosclerosis [25–29]. Furthermore, catalase and its derivatives inhibit cisplatin-induced nephrotoxicity, thus improving the efficiency of cisplatin to treat solid tumors [30].

In this study, bovine liver catalase (BLC) interactions with [Pd(dach)(FIP)]$(NO_3)_2$ was investigated kinetically and thermodynamically. BLAST (Basic Local Alignment Search Tool) tool results indicated that BLC has 95% similarities and 91% identities with human catalase, which is why selected as a model protein instead of human liver catalase in our spectroscopic study [31].

By UV–vis spectrophotometer, changes in kinetic parameters like enzyme activity, Michaelis menten constant (K_m), maximum velocity (v_{max}) and catalytic rate constant (k_{cat}) was determined. Then, the inhibitory behavior of palladium complex was monitored to find out the inhibition type. Regarding binding and thermodynamic parameters, fluorescence and circular dichroism (CD) spectrophotometry methods were applied at two temperatures of 25 (room temperature) and 37 °C (physiologic temperature). This way, binding sites (n), binding constants (K), Stern-volmer constants (K_{SV}), enthalpy changes ($\Delta H°$), entropy changes ($\Delta S°$), Gibbs free energy changes ($\Delta G°$) and secondary structure contents (α-helix, β-sheet and random coil) of BLC were calculated at both mentioned temperatures.

2. Experimental

2.1. Materials

BLC (C9322) was purchased from Sigma–Aldrich Company. Hydrogen peroxide 30%, and sodium phosphate salts were obtained from Merck Company. [Pd(dach)(FIP)]$(NO_3)_2$was synthesized in our laboratory according to previous reports [4].

2.2. Preparation of solutions

2mM aqueous solution of [Pd(dach)(FIP)]$(NO_3)_2$ was prepared in distilled water with gentle heating and stirring. Moreover, with a diluted 1000 mM H_2O_2 solution, a calibration curve was plotted. Therefore the hydrogen peroxide concentration detected during the enzymatic reaction [32].

2.3. Kinetic investigations

To determine catalase activity with 1 nM concentration, a GBC Cintra UV–vis 101 Spectrophotometer was used at 240 nm. According to Hugo method [33], 50 mM phosphate buffer (pH 7.0) plus 30 mM H_2O_2 was present in the reaction mixture. This method suggests that the H_2O_2 concentration must be in 0.3–2.5 K_m range. To investigate the palladium complex effects on the enzyme activity, in the fixed concentrations of H_2O_2 (1 nM) and BLC (30 mM), the various concentrations of complex (0–40 μM) were applied at 25 °C.

2.4. Fluorescence measurements

A Varian Spectrofluorometer, Cary eclipse model was utilized to monitor the Intrinsic fluorescence spectra of enzyme and changes in tertiary structure of BLC (0.8 μM) at 25 and 37 °C, in the Absence and presence of different concentrations of [Pd(dach)(FIP)]$(NO_3)_2$ (0–40 μM). The Trp residues was excited at 290 nm at 25 and 37 °C in a quartz cell with 1 cm path length and the spectra were recorded from 300 to 500 nm.

2.5. ANS (8-anilino-1-naphthalenesulphonic acid) fluorescence measurements

Hydrophobic behavior of enzyme was studied by ANS fluorescence spectra which were recorded at 25 and 37 °C temperatures with a Varian Spectrofluorometer, Cary eclipse model.

Protein and ANS concentrations in a quartz cell with 1 cm path length were set to 0.8 μM and 300 mM, respectively. In the excitation wavelength of 365 nm, the emission spectra were recorded from 400 to 700 nm after 10 min incubation in the absence and presence of different concentrations of palladium compound (0–44 μM).

2.6. Circular dichroism measurements

Changes in secondary structure of BLC were monitored instrumentally by Circular dichroism (CD) technique in the far-UV regions (190–260 nm) with a J810Jasco Spectropolarimeter in a 1-mm cell at 25 °C. CD software of CDNN was applied to statistical interpretation of changes in the secondary structure content of catalase (1.2 μM). All measurements was carried out in the Absence and presence of various concentrations of Pd(II) complex (0–66 μM) in 50 mM phosphate buffer (pH 7.0) at 25 °C.

3. Results and discussion

3.1. Kinetic study of [Pd(II) complex effects on BLC function

Changes in light absorbance at 240 nm were applied to track hydrogen peroxide concentration and also enzyme activity during the experiments. The initial concentration of H_2O_2 was set to 30 mM [33].

Moreover, the final concentration of enzyme in this kinetic assay was fixed at 1 nM.

By increasing the Pd(II) complex concentrations up to 30 μM, enzyme activity was slowly reduced to 65% and then unchanged in higher concentrations which is depicted in Fig. 2.

According our previous reports, compared to mostly similar complex of platinum, [Pt(FIP)(phen)]$(NO_3)_2$, which reduced the activity to