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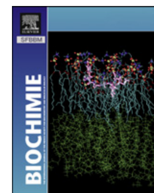

Research paper

Interaction between lanthanum ion and horseradish peroxidase *in vitro*

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ABSTRACT

The interaction between lanthanum ion (La^{3+}) and horseradish peroxidase (HRP) *in vitro* was investigated using a combination of biophysical and biochemical methods. When the molar ratio of La^{3+} and HRP is low, it was found that the interaction between La^{3+} and HRP mainly depends on the electrostatic attraction, van der waals force and hydrogen bond etc. Thus, the interaction is weak and the La–HRP complex cannot be formed *in vitro*. As expected, the interaction can change the conformation of HRP molecule, leading to the increase in the non-planarity of the porphyrin ring in the heme group of HRP molecule, and then in the exposure degree of the active center, Fe(III) of the porphyrin ring of HRP molecule. Therefore, the catalytic activity of HRP for the H_2O_2 reduction is improved. When the molar ratio of La^{3+} and HRP is high, La^{3+} can strongly coordinate with O and/or N in the amide group of the polypeptide chain of HRP molecule, forming the La–HRP complex. The formation of the La–HRP complex causes the change in the conformation of HRP molecule, leading to the decrease in the non-planarity of the porphyrin ring in the heme group of HRP molecule, and then in the exposure degree of the active center, Fe(III) of the porphyrin ring of HRP molecule. Thus, the catalytic activity of HRP for the H_2O_2 reduction is decreased comparing with that of HRP in the absence of La^{3+}. The results can provide some references for understanding the interaction mechanism between trace elements ions and peroxidase in living organisms.

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1. Introduction

Trace elements, especially heavy metals and metalloids are widely used in many fields, such as functional materials, catalysts and additives [1–5]. It has been demonstrated that some trace elements at the low concentration could improve the growth of animals and plants, but at the high concentration, they would become toxic [6–10]. It has been reported that rare earth elements (REEs), a kind of trace elements, possess the same effect on living organisms as trace elements mentioned above. It was found that REEs exert the positive and negative effects on living organisms by regulating the metabolism of free radicals, such as superoxide radicals, hydrogen peroxide (H_2O_2) and hydroxyl radicals [8]. It was well known that the antioxidant enzymes play a key role in the scavenging of the free radicals in living organisms [11]. Thus, it is important to study the effect of REEs on the structure and function of those enzymes in living organisms.

Peroxidases containing the heme group are found in prokaryotic and eukaryotic organisms. They are typical antioxidant enzymes. HRP from horseradish (*Armoracia rusticana*) is an example of the plant proteins and enzymes containing the heme group. It is often used for the investigation of the interaction between trace elements and peroxidase. Seven isoenzymes of HRP have been identified. Among them, c isoenzyme of HRP (HRPc) is the most abundant and has been successfully isolated, purified and characterized [12]. The molecular structure of HRPc has been solved and its molecular weight is about 44 kD (Fig. 1) [12]. It has 26 aromatic residues (5 Tyrosine, 1 Tryptophan and 20 Phenylalanine). There are four disulphide bridged between cysteine (Cys) residues 11–91, 44–49, 97–301 and 177–209 and a buried salt bridge between Asp99 and Arg123 in HRP molecule. HRPc contains two different types of metal center, iron (III) in the heme group and two calcium (II) (Fig. 1). They are essential for the structural and functional integrity of HRP [12,13]. It was found from our previous study that heavy REEs, Tb(III) can decrease the bioactivity of HRP both *in vitro* and *in vivo* with the formation of the Tb–HRP complex [14]. However, the biochemical behavior of the light REEs in plant is different from the heavy REEs [15,16]. The light REEs normally

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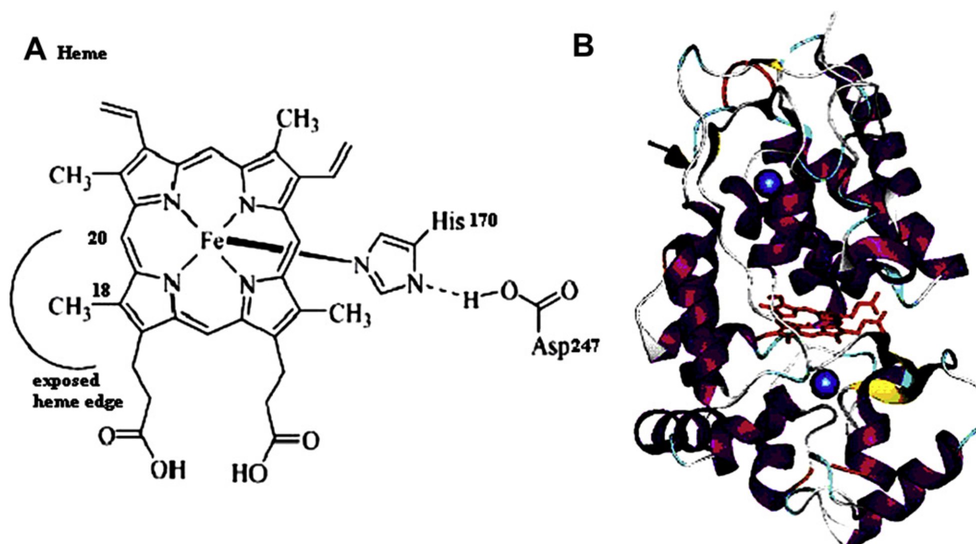

Fig. 1. The heme structure and secondary structure of HRPc, reported in reference 12.

possess relatively big ionic radii and relatively low stability constant for naturally relevant complexes, comparing with the heavy REEs [17]. Therefore, it is necessary to investigate the interaction mechanism between the light REEs and HRP.

Lanthanum (La) is one of trace elements and REEs naturally found in the environment but also used in the production of several fertilizers [1,18]. In this work, the interaction between La^{3+} and HRP in the simulated physiological solution were investigated using a combination of biophysical and biochemical methods, such as cyclic voltammetry (CV), atomic force microscopy (AFM), ultraviolet–visible (UV–vis) absorption spectroscopy, circular dichroism (CD), high performance liquid chromatography (HPLC), matrix-assisted laser desorption/ionization time-of-flight mass spectra (MALDI-TOF/MS), inductively coupled plasma mass spectrometry (ICP-MS), X-ray photoelectron spectroscopy (XPS) and molecular dynamics simulation.

2. Materials and methods

2.1. Chemicals

$LaCl_3$ was purchased from Aldrich Chemical Co. and its purity was higher than 99.99%. Lyophilized powder of HRP was purchased from Sigma Chemical Co. ($R_Z = A_{403}/A_{275} \geq 2.5$) and was further purified before every experiment according to the literature [19]. The concentration of HRP solution was measured according to the reference [19]. Cys was dissolved in the distilled water to obtain 1 mM solution. All glassware used in the following procedures were cleaned with freshly prepared HNO_3 + HCl solution with 3:1 volume ratio and then rinsed thoroughly with twice-distilled water. The spherical colloidal Au nanoparticles were prepared with the citrate reduction of $HAuCl_4$ in the aqueous solution [20]. The Au colloids were prepared by adding the sodium citrate solution to a boiling $HAuCl_4$ solution. By changing the molar ratio of $HAuCl_4$ and sodium citrate, the different diameters of the Au colloids were obtained. In the study, the average diameter of the Au colloid was about 24 nm, which was measured using transmission electron microscopy. All other chemicals were of analytical grade. The simulated physiological solution was prepared according to the references [21–23]. It consists of the Krebs solution modified with 5 mM Tris–HCl buffer (pH 6.9), Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-. The ionic strength of the simulated physiological solution was 0.1 M. All experiments were conducted in the simulated physiological solution.

2.2. Measurement methods

A 1.26×10^{-5} M HRP solution containing different concentration of La^{3+} in the simulated physiological solution was equilibrated for 12 h at 25 °C. Then, the mixed solution of HRP and La^{3+} was used to measure the catalytic activity of HRP. The catalytic activity was determined with the increase per minute in the absorbance at 414 nm due to the oxidation of 0.01 mM 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with 0.002 mM H_2O_2 [24]. The relative catalytic activity of HRP was expressed as the ratio of the catalytic activities of HRP with and without La^{3+}.

The measurements of the cyclic voltammetry were performed with an EG&G PAR Model 273 Potentiostat/galvanostat with a traditional three-electrode electrochemical cell. A platinum wire and the saturated calomel electrode (SCE) were used as the auxiliary and reference electrodes, respectively. All potentials in this paper were quoted with respect to SCE. The modified Au electrode (Au/Cys/Au electrode) was used as the working electrode. The working electrode was prepared according to the method reported by Gu [25]. Its apparent surface area is about 0.031 cm^2. After the La^{3+} solution was added into the 1.26×10^{-5} M HRP solution and the mixture solution was stirred and equilibrated for 12 h at 4 °C, the electrochemical measurements were carried out. The electrolyte solution was purged with the pure nitrogen prior to the electrochemical experiments. All experimental temperature was 25 ± 0.4 °C.

The AFM measurements were performed with a Digital NanoScope III a system (Santa Barbara, USA) in the tapping mode at room temperature. The sample for the AFM measurement was prepared as follows. The Au/Cys/Au electrode was dipped into 1.26×10^{-5} M HRP solution with the different concentration of La^{3+} for 12 h at 4 °C. After being dried, it was used for the AFM measurement.

The measurements of the UV–vis absorption spectroscopy were performed on a Cary 50 Probe (Varian, Australia) spectrophotometer in a 1.0-cm path length quartz cell. The solution without HRP was used as the reference solution.

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