Asian Journal of Chemistry; Vol. 27, No. 7 (2015), 2365-2368

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17832

Promotion Effect of La^{3+} on the Catalytic Activity of Horseradish Peroxidase *in vitro*

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Received: 16 April 2014; *Accepted*: 24 June 2014; *Published online*: 30 March 2015; AJC-17034

In this paper, the promotion effect of La^{3+} on the catalytic activity of horseradish peroxidase was investigated *in vitro* by using the combination of cyclic voltammetry, ultraviolet-visible, atomic force microscopy, high performance liquid chromatography and inductively coupled plasma mass spectrometry. The results indicated that the low concentration of La^{3+} could promote the catalytic activity of horseradish peroxidase by changing the secondary structure or the conformation of horseradish peroxidase and loosing the tertiary structure of the horseradish peroxidase and/or acting as an activator. La^{3+} could lead to the decrease in planarity of the porphyrin cycle in heme group, thus the exposure extent of heme Fe(III) is increased, the electron transfer is convenient and thus horseradish peroxidase catalytic activity is promoted, but La^{3+} did not bind with horseradish peroxidase. Therefore, there exists a weak interaction between La^{3+} and horseradish peroxidase *in vitro*. The result could provide a reference point on better understanding in agricultural application of rare earth elements.

Keywords: La^{3+}, Horseradish peroxidase, Catalytic activity, Conformation change.

INTRODUCTION

Rare earth elements are widely applied in various fields because of their special electronic structure, physical and chemical properties[1]. In China and other countries, rare earth elements have been used in the agriculture field as the additives of fertilizers to promote plant growth, which is a Chinese government behavior. From the beginning of 20th century, the biological functions of rare earth elements in living organisms have been studied extensively[2-4]. However, a lot of researches pay attention to the biochemical and physiological effects of rare earth elements on plant[5], few relates to the effect mechanism and chemical process of rare earth elements on the plant biological function. It was reported that the protective enzymatic systems, such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) are very sensitive to the rare earth elements. Our group have investigated that Tb^{3+} could affect peroxidase activity and conformation both *in vivo*[6] and *in vitro*[7] and the effect mechanism was proposed, *i.e.*, formation of Tb^{3+} and peroxidase complexes.

In this paper, we studied the effect of La^{3+} (one of the light rare earth elements and widely used in agriculture fields) on the catalytic activity of horseradish peroxidase. We hope that this study could provide some references on further understanding the physiological and biochemical functions of rare earth elements on the molecular level.

EXPERIMENTAL

Lyophilized powder of horseradish peroxidase ($R_Z = A_{403}/A_{275} \geq 2.5$) was purchased from Sigma Co. and was further purified before used. The concentration was determined spectrophotometrically using the extinction coefficient of 102 mM^{-1} cm^{-1} at 403 nm[8]. Other chemicals were of analytical reagents. The simulated physiological solution was prepared by known method[6]. All experiments were conducted in the simulated physiological solution.

The catalytic activity of horseradish peroxidase with different concentration of La^{3+} in the simulated physiological solution was performed by reported method[9]. The cyclic voltammetry, atomic force microscopy, HPLC and ICP-MS measurements were performed according to previous reports[7,10].

UV-visible absorption spectra were obtained using a Perkin-Elmer Lambda 16 UV-visible recording spectrophotometer with 0.5 cm path length cell. The relational physiological solution without La^{3+} was used as the reference solution. Absorbance difference spectra were obtained between 190 and 700 nm.

RESULTS AND DISCUSSION

Effect of La^{3+} on the relative catalytic activity of horseradish peroxidase: Fig. 1 showed the effect of different concentrations of La^{3+} on the horseradish peroxidase relative catalytic activity in the simulated physiological solution. It can be seen that horseradish peroxidase relative catalytic activity is firstly stimulated in the presence of low La^{3+} concentration and then horseradish peroxidase relative catalytic activity is decreased with increasing of the La^{3+} concentration. When the molar ratio of La^{3+} and horseradish peroxidase was less than 5, the relative catalytic activity of horseradish peroxidase increased with the increasing of La^{3+} concentration. When the molar ratio of La^{3+} and horseradish peroxidase was 3, the relative catalytic activity of horseradish peroxidase increased about 13.1 %. When the molar ratio of La^{3+} and horseradish peroxidase was 5, horseradish peroxidase relative catalytic activity reached the highest point. The molar ratio of La^{3+} and horseradish peroxidase increasing more than 5, horseradish peroxidase relative catalytic activity decreased rapidly, when $[La^{3+}]/[HRP] = 10$, horseradish peroxidase relative catalytic activity decreased by 12.2 %. These results indicated that the effect of La^{3+} on the relative catalytic activity of horseradish peroxidase displayed "hormesis effect"[11]. In order to obtain the promotion mechanism of La^{3+} on the relative catalytic activity of horseradish peroxidase, $[La^{3+}]/[HRP] = 3$ was used to discuss the promotion effect of La^{3+} on horseradish peroxidase relative catalytic activity *in vitro*.

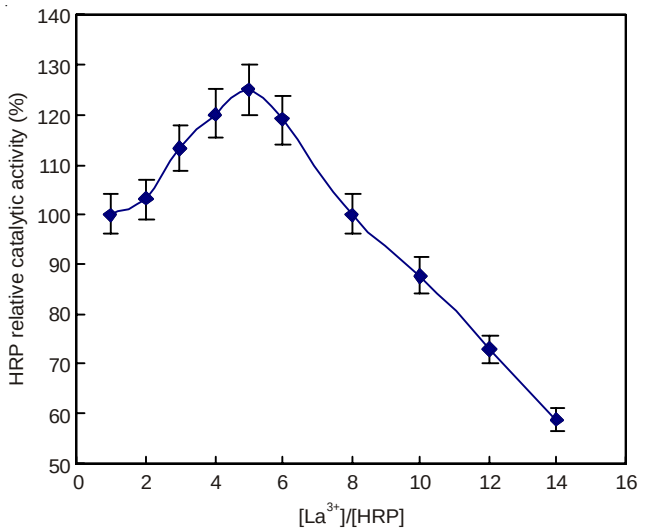

Fig. 1. Effect of La^{3+} concentration on the relative catalytic activity of horseradish peroxidase in the simulated physiological solution

Effect of La^{3+} on the electrochemical property of horseradish peroxidase: Fig. 2A showed the cyclic voltammograms of the 1.26×10^{-5} M horseradish peroxidase in the simulated physiological solution with 3.78×10^{-5} M La^{3+} at Cys-Au/GC electrode. In the absence of La^{3+}, a pair of redox peaks is located at -0.307 and -0.400 V, which is attributed to the direct electrochemical reaction of the heme group of horseradish peroxidase (Fig. 2A, curve a)[12]. The difference between the cathodic and anodic peak potentials, ΔEp is 93 mV. The anodic peak current (I_{pa}) is 0.108 μA, which is almost equal to the cathodic peak current (I_{pc}), indicating that horseradish peroxidase can undergo a direct and quasi-reversible electrochemical reaction at the Cys-Au/GC electrode[13]. The formal redox potential, $E^{o'}$ is -0.354 V. It is in the good agreement with former reports[14]. In the presence of La^{3+}, a pair of redox peaks is at -0.331 and -0.398 V and ΔEp is 67 mV. I_{pa} is almost equal to I_{pc}, indicating that horseradish peroxidase also undergoes a direct and quasi-reversible electrochemical reaction at the Cys-Au/GC electrode in the simulated physiological solution with La^{3+}. $E^{o'}$ is -0.365 V, which is more negative than that of in the absence of La^{3+}. The negative shift of $E^{o'}$ illustrated the decrease in the hydrophobicity of the microenvironment of the heme group of horseradish peroxidase. Furthermore, I_{pa} of horseradish peroxidase is 0.122 μA, which is increased by 11.4 % comparing with that for horseradish peroxidase in the absence of La^{3+}. The above results demonstrated that the electrochemical reaction activity of horseradish peroxidase is promoted by La^{3+}.

Fig. 2B showed the cyclic voltammograms of 1.26×10^{-5} M horseradish peroxidase in the simulated physiological solution without (a) and with La^{3+} (b) in the presence of 0.42 μM H_2O_2 at the Cys-Au/GC electrode. In the absence of La^{3+}, a marked reduction peak of H_2O_2 appears and its I_{cat} is 1.20 μA (Fig. 2B, curve a). In the presence of La^{3+}, the peak current of H_2O_2 reduction is significantly increased to 1.42 μA (Fig. 1B, curve b). The phenomenon indicated that La^{3+} can indeed promote the electrocatalytic activity of horseradish peroxidase for the reduction of H_2O_2 (Table-1).

TABLE-1
ELECTROCHEMICAL DATA OF HORSERADISH PEROXIDASE WITHOUT AND WITH La^{3+} IN THE SIMULATED PHYSIOLOGICAL SOLUTION

Composition	$E^{o'}$ (mV)	I_{pa} (μA)	E_{pa} (mV)	E_{pc} (mV)	ΔE_p (mV)	$I_{cat}(H_2O_2)$ (μA)
$[La^{3+}]/[HRP] = 0$	-354	0.108	-307	-400	93	1.20
$[La^{3+}]/[HRP] = 3$	-365	0.122	-331	-398	67	1.42

Atomic force microscopy images: Fig. 3a showed the atomic force microscopy images of horseradish peroxidase assembled on the surface of the gold nanoparticles. It was found from Fig. 3a that horseradish peroxidase was uniformly distributed on the surface of the gold nanoparticles. The average diameter of the horseradish peroxidase molecules is about 60 nm. In the presence of 3.78×10^{-5} M La^{3+}, the atomic force microscopy image is changed (Fig. 3b), the average diameter of the horseradish peroxidase molecules is increased to about 66 nm. This may be due to the looseness of the horseradish peroxidase molecules on electrode surface with low concentration of La^{3+}. Horseradish peroxidase has a very extensive hydrogen-bonding network in the proximal and distal regions of the heme group. The distal region is connected to the proximal region by a hydrogen-bonding network. It was known from the above results that La^{3+} might interact with some amide groups in horseradish peroxidase, causing the looseness of the structure of the horseradish peroxidase molecule and thus, the average diameter of the horseradish peroxidase molecules is increased. Obviously, the looseness of the horseradish peroxidase molecules would increase the exposure extent of heme active center in horseradish peroxidase significantly, thus increase the catalytic and electrochemical activities of horseradish peroxidase.

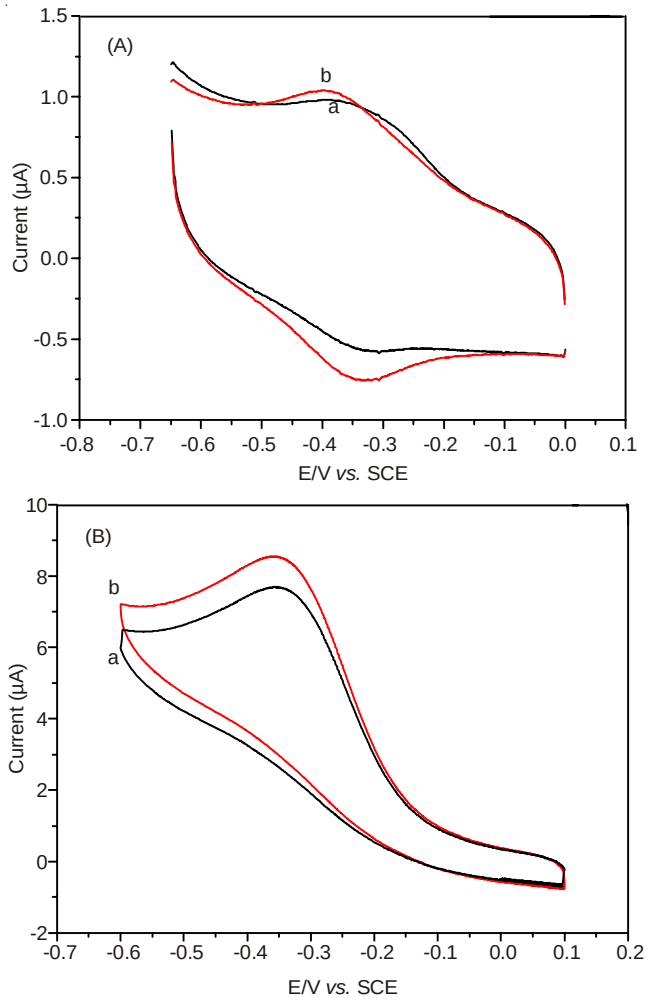

Fig. 2. (A) Cyclic voltammetry of horseradish peroxidase without and with La^{3+} at the Au/Cys/GC electrode in the simulated physiological solution, scanning rate: 100 mV s^{-1}. [La^{3+}]/[HRP]: (a)0; (b)3; (B) Cyclic voltammetry of horseradish peroxidase without and with La^{3+} in catalyzing H_2O_2 at the Au/Cys/GC electrode in the simulated physiological solution, scanning rate: 100 mV s^{-1}. [La^{3+}]/[HRP]: (a)0; (b)3

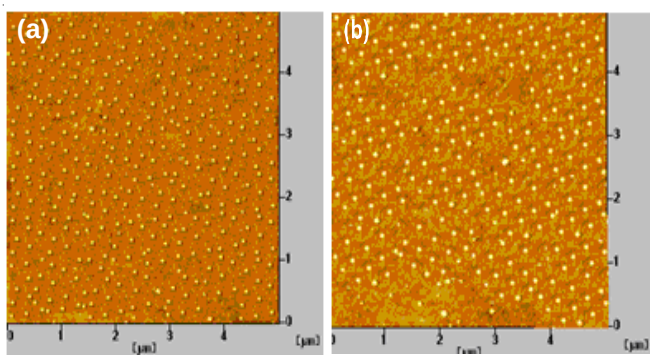

Fig. 3. Atomic force microscopy images of horseradish peroxidase without and with La^{3+} deposited on the surface of the gold nanoparticles, [La^{3+}]/[HRP]: (a)0; (b)3

UV-visible spectra: Fig. 4 showed the UV-visible spectra of 1.26×10^{-5} M horseradish peroxidase without (a) and with 3.78×10^{-5} M La^{3+} (b). The peak near 200 nm is due to π-π* transition of amide in peptide chain, the Soret band around 399 nm (γ-band) is attributed to π-π* transition of heme porphyrin cycle. It was clearly showed that after the addition

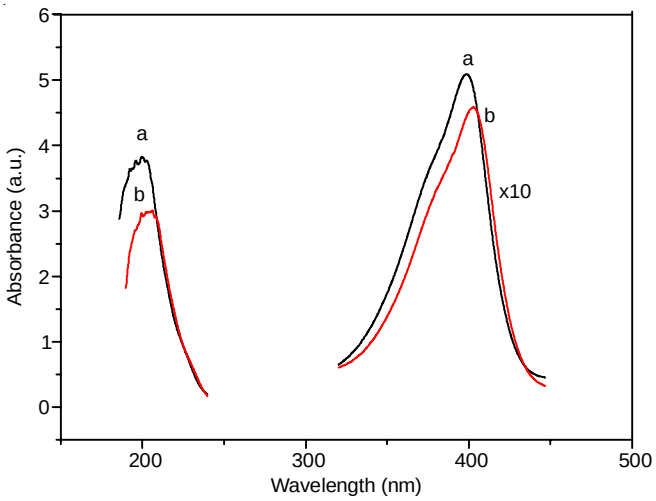

Fig. 4. UV-visible spectra of horseradish peroxidase without and with La^{3+} in the simulated physiological solution, [La^{3+}]/[HRP]: (a)0; (b)3

of La^{3+} ion (curve b), the peak near 200 nm was red shifted to 204 nm and the intensity was decreased about 22.7 %, the Soret band was red shifted to 403 nm and the intensity was decreased about 11.4 % comparing to curve a, respectively. The results indicated that there was an interaction between La^{3+} ion and horseradish peroxidase and La^{3+} ion would mainly have an influence on the amino acid residues of horseradish peroxidase. The red shift and intensity decrease at the Soret band suggested that the planarity and π-π* transition energy of the porphyrin cycle in the heme group is decreased[15], thus, the exposure extent of heme active center is increased, electron transfer become easier, so the catalytic activity of horseradish peroxidase is promoted.

HPLC and ICP-MS: Fig. 5 showed the HPLC elution profiles of 1.26×10^{-5} M horseradish peroxidase without (a) and with 3.78×10^{-5} M La^{3+} (b). It was observed from Fig. 5A that only one elution peak appears at 8.8 min, indicating that the native horseradish peroxidase is eluted at 8.8 min. Meanwhile, in Fig. 5B there was also one elution peak appears at 8.8 min, indicating that after interaction of La^{3+} and horseradish peroxidase, no new substance was produced. The contents of the metal cations in 1 mol horseradish peroxidase without and with La^{3+} collected from the elution profile at 8.8 min were determined by ICP-MS, respectively and listed in Table-2. The results indicated that there were about 2 molars Ca^{2+} in 1 mol horseradish peroxidase molecules without La^{3+}, which is similar to the content of metals in commercial HRPc because one HRPc molecule contains two Ca^{2+} ions and one Fe^{3+} ion in protoporphyrin IX. It also displayed that Ca^{2+} and Fe^{3+} in horseradish peroxidase with the low concentration of La^{3+} were equal to that of in horseradish peroxidase without La^{3+} and no La^{3+} were detected in the two samples. However, in previous research, horseradish peroxidase interacted with high concentration of La^{3+}, a new substance La-HRP complex was formed[10]. The results indicated that the low concentration of La^{3+} could not coordinate with horseradish peroxidase, it might be a weak or noncovalent interaction between La^{3+} and horseradish peroxidase, La^{3+} mainly changes some factors in maintaining and stabilizing the structure of horseradish peroxidase protein, such as electrostatic interaction, hydrogen bond,

TABLE-2
CONTENTS OF Fe^{3+}, Ca^{2+} and La^{3+} in 1 μM HORSERADISH PEROXIDASE SOLUTIONS WITHOUT AND WITH La^{3+}

Composition	Fe^{3+} (μM)	Ca^{2+} (μM)	La^{3+} (μM)
$[La^{3+}]/[HRP]=0$	0.98 ± 0.03	2.02 ± 0.02	0.00 ± 0.02
$[La^{3+}]/[HRP]=3$	1.01 ± 0.02	1.99 ± 0.04	0.00 ± 0.01

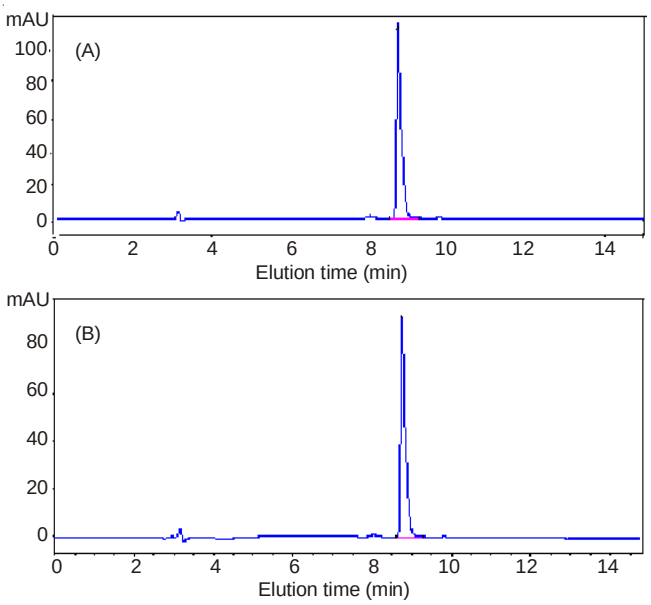

Fig. 5. HPLC elution profiles of horseradish peroxidase without and with La^{3+} in the simulated physiological solution, $[La^{3+}]/[HRP]$: (A)0; (B)3

hydrophobic interaction, van der Waals interaction. The weak interaction might disturb the secondary or the tertiary structure of horseradish peroxidase and thus increased horseradish peroxidase catalytic activity.

Conclusion

In this work, it was found that when La^{3+} interacts with horseradish peroxidase, La^{3+} can interact with the amide groups of the peptide of the horseradish peroxidase molecules leading to the conformation change of horseradish peroxidase. This change would decrease the planarity of porphyrin cycle in heme group and increase the exposure extent of heme active center, Fe^{3+}. The increase in the exposure extent of heme active center would increase the catalytic and electrochemical activities of horseradish peroxidase.

ACKNOWLEDGEMENTS

The work is supported by the financial support of the National Natural Science Foundation of China (No.41201526, 41301320), the Promotive Research Fund for Young and Middle-aged Scientists of Shandong Province (No. BS2010SW003), the Program for Science and Technology of Shandong Province University (No. J12LM59) and the Shandong Province Youth Education Science Programs-College Student Academic Subject (14BSH149).

REFERENCES

1. H. Liu, P. He, Z. Li, Y. Liu and J. Li, *Electrochim. Acta*, **51**, 1925 (2006).
2. Y.-W. He and C.-S. Loh, *Plant Sci*., **159**, 117 (2000).
3. H. Wu, X. Zhang, P. Liao, Z. Li, W. Li, X. Li, Y. Wu and F. Pei, *J. Inorg. Biochem*., **99**, 2151 (2005).
4. J.Z. Ni, Bioinorganic Chemistry of Rare-Earth Elements, Science Press, Beijing, pp. 18 (1995). (in Chinese).
5. C. Liang, X. Huang, W. Tao and Q. Zhou, *J. Rare Earths*, **24**, 364 (2006).
6. S.F. Guo, R. Cao, A.H. Lu, Q. Zhou, T.H. Lu, X.L. Ding, C.J. Li and X.H. Huang, *J. Biol. Inorg. Chem*., **13**, 587 (2008).
7. S.F. Guo, Q. Zhou, T.H. Lu, X.L. Ding and X.H. Huang, *Spectrochim. Acta A*, **70**, 818 (2008).
8. K. Chattopadhyay and S. Mazumdar, *Biochemistry*, **39**, 263 (2000).
9. D. Pahari, A. Patel and D. Behere, *J. Inorg. Biochem*., **60**, 245 (1995).
10. S.F. Guo, L.H. Wang, A.H. Lu, T.H. Lu, X.L. Ding and X.H. Huang, *Spectrochim. Acta part A*., **75**, 936 (2010) .
11. K. Wang, R. Li, Y. Cheng and B. Zhu, *Coord. Chem. Rev*., **190-192**, 297 (1999).
12. J.L. Tang, J.G. Jiang, Y.H. Song, Z.Q. Peng, Z.Y. Wu, S.J. Dong and E.K. Wang, *Chem. Phys. Lipids*, **120**, 119 (2002).
13. Y. Zhang, P. He and N. Hu, *Electrochim. Acta*, **49**, 1981 (2004).
14. D. Sun, C. Cai, X. Li, W. Xing and T. Lu, *J. Electroanal. Chem*., **566**, 415 (2004).
15. H.J. Jiang, X.H. Huang, X.F. Wang, X. Li, W. Xing, X.L. Ding and T.H. Lu, *J. Electroanal. Chem*., **545**, 83 (2003).