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Activity Regulation of Lignin Peroxidase from *Phanerochaete chrysosporium* in Nonionic Reversed Micellar Medium

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Abstract: The activity of lignin peroxidase (LiP) in reversed micelles of polyoxyethylene lauryl ether (Brij30) changed with the molar ratio of water to the surfactant and the denaturant concentration of guanidinium chloride. At low water contents the activity of LiP could be enhanced by the denaturant at moderate concentration. This phenomenon, together with the spectral characteristics of the intrinsic fluorescence of LiP, suggested that the conformation of the active center of LiP was flexible.

Keywords: Lignin peroxidase, reversed micelle, denaturant, activation.

The reversed micelles, aggregate of surfactant molecules, provide thermodynamically stable aqueous microenvironments in the organic media. One of their most important properties is that their dimension can be easily varied by changing the molar ratio of water to surfactant (ω_0). Because of the distinct amphiphilic property, the reversed micelles have been used as media for biocatalyzed reactions, especially for the biotransformation of hydrophobic substrates catalyzed by hydrophilic enzymes. An enzyme hosted in a reversed micelle is usually catalytically active, due to the similarity in the structure of surfactant aggregates to a cell membrane. For a given surfactant, the activity of an enzyme can be altered either by varying ω_0 or by adding a denaturant. A reversed micelle is therefore a good model for studying the relationship between enzyme activity and conformation flexibility[1].

So far, the reversed micelles used for above-mentioned target are mostly formulated with ionic surfactants, such as sodium bis(2-ethylhexyl)sulphosuccinate (AOT), cetyltrimethylammonium bromide (CTAB), *etc.*[1-3]. The head groups of these surfactants are small (rigid) and electrically charged. To alleviate the restriction imposed by the interface on the conformation of the enzyme entrapped in reversed micelles, a reversed micelle formulated with a nonionic surfactant of big head group was used in this paper so as to further understand the phenomenon that some enzyme entrapped in reversed micelles could be activated by denaturants.

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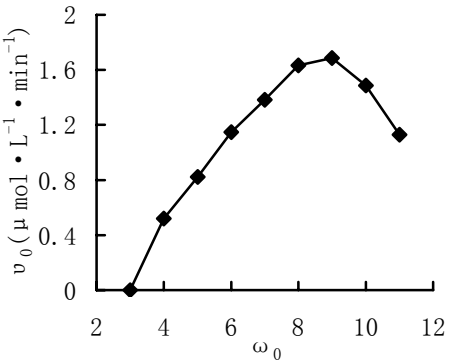
Figure 1 Effect of ω_0 on the activity of LiP in Brij30 reversed micelles



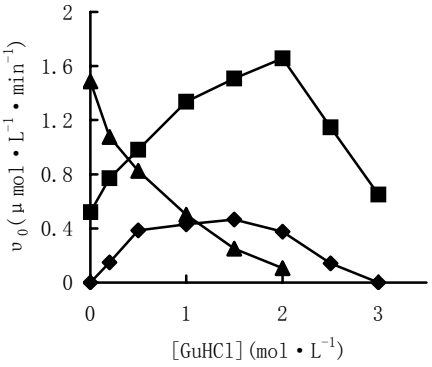
T=20℃, cyclohexane 2.8 mL, [Brij30]=600 mmol· L^{-1}, [VA]=10 mmol· L^{-1}, [LiP]=0.38 μmol· L^{-1}, [H_2O_2]=0.067 mmol· L^{-1}. Preparation of the reversed micelles and assay of LiP activity see Reference and Notes[9,10].

Lignin peroxidase (LiP) is an extracellular heme protein secreted by white rot fungus. It plays a key role in lignin biodegradation[4]. Recent studies also show that it can degrade environmental pollutants[5-7]. Therefore studies on LiP are of great significance to the resources and environment.

The catalytic and kinetic properties of LiP hosted in reversed micelles are rarely studied. It was reported that LiP from *Streptomyces viridosporus* T7A was catalytically active in AOT reversed micelles, but it did not exhibit catalytic activity in CTAB reversed micelles[8]. Here we found that LiP from *Phanerochaete chrysosporium* showed catalytic activity in the reversed micelles formed by a nonionic surfactant polyoxyethylene (4) lauryl ether (Brij30) without any co-surfactant. And a typical bell-shaped curve was observed for the activity of LiP as a function of ω_0 (**Figure 1**). The maximum activity occurred at ω_0=10, which was defined as the optimum ω_0.

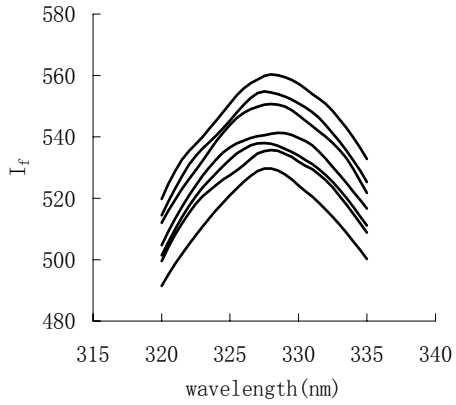
Guanidinium chloride (GuHCl) is a well-known denaturant of proteins. At ω_0 = 10 (larger than the optimum value), GuHCl has an inactivating effect on the LiP activity, which is very similar to that in aqueous medium. At lower water contents, however, the effect of GuHCl on the LiP activity is related to the concentration of GuHCl as well as to ω_0 (**Figure 2**). At ω_0=4 (lower than the optimum ω_0), the activity of LiP increased first and then decreased obviously with the increase of GuHCl concentration with a inflection point at 2 mol • L^{-1}. In aqueous medium LiP could not tolerate such high concentration of GuHCl (LiP was completely inactivated by GuHCl at a concentration of 0.5 mol • L^{-1}). Based on the relationship between enzyme activity and its conformation, we deduce that the conformation of LiP in the reversed micelles is perturbed by GuHCl.

Figure 2 Effects of GuHCl concentration on the activity of LiP in Brij30 reversed micelles



(◆) ω_0=3, (■) ω_0=4, (▲) ω_0=10. Other conditions are the same as in **Figure 1.**

Figure 3 Emission spectra of LiP in Brij30 reversed micelles (ω_0 = 4) with and without GuHCl



The concentrations of GuHCl corresponding to the curves, from up downwards, are 0, 0.2, 0.5, 1, 1.5, 2, 3 mol· L^{-1}, respectively; cyclohexane 2.8 mL; [Brij]=600 mmol· L^{-1}; [LiP]=0.38 μmol· L^{-1}. Instrumental conditions see reference and notes[11].

The intrinsic fluorescence of a protein can, in some sense, reflect its conformation change caused by denaturants[1-3]. **Figure 3** shows the emission spectra of LiP in the reversed micelles with and without GuHCl. Except for emission intensity, there is no obvious difference in the spectra recorded at different GuHCl concentrations. Similar result was reported by Shoshani *et al.*[1-3]. It is believed that the conformation of the active site is more flexible and more sensitive to denaturant than the molecule as a whole[1,12] and that small change in the conformation of the active site of the enzyme can cause an obvious change in the activity. Compared with AOT and CTAB, which have rigid head groups, the interface of Brij30 reversed micelles seems to have small restriction on the conformation of LiP. So we speculated that the perturbation of the conformation of LiP at the active site induced by GuHCl should be responsible for the change of activity of LiP in Brij30 reversed micelles. In other words, the activation was due to subtle conformation changes at the active site of LiP.

Acknowledgments

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9. *Preparation of Brij30 reversed micelles:* Brij30 was dissolved in cyclohexane to obtain a Brij30 solution with a final concentration of 0.6 mol· L^{-1}, then an aliquot of citric buffer solution (0.1 mol· L^{-1}, pH=2.2) was injected into it (ω_0 was calculated based on the water from the GuHCl, LiP and H_2O_2 solutions prepared with the citric buffer as well as that from the buffer). The mixture was shaken gently until a clear solution was obtained.
10. *Assay of LiP activity:* The activity of LiP was evaluated as an initial reaction rate of the LiP-catalyzed oxidation of VA, which was monitored by following the change in absorbance at 310 nm due to formation of veratraldehyde (its molar absorptivity was 9.3×10^3 L•mol^{-1}•cm^{-1}). The procedure for the measurements was as follows: The reversed micelle solution containing VA (VA was directly dissolved into the reversed micelles) was pipetted into a 1 cm long cuvette, then a aliquot of LiP solution was added and shaken until the mixture was clear, the oxidation reaction was then initiated by addition of H_2O_2 solution. The plot of absorbance (A) at 310 nm *versus* the reaction time (t) was recorded with the Shimadzu UV-240 spectrophotometer, and the initial rate in μmol•L^{-1}•min^{-1} was calculated from the initial slope through an absorbance/concentration conversion. For the study of the effect of GuHCl on the LiP activity, the required concentration of GuHCl in reversed micelles (calculated based on the volume of the aqueous phase, other components were based on the whole volume of the micellar system) was obtained by injecting a certain amount of GuHCl stock solution (5 mol· L^{-1}), and GuHCl was added before adding H_2O_2.
11. *Measurement of intrinsic fluorescence:* The intrinsic fluorescence of LiP was measured on a Perkin Elmer LS-55 luminescence spectrometer with an excitation wavelength of 278 nm. The excitation slit was 10 nm and the emission slit 8 nm. The scan speed was 1500 nm· min^{-1}.
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