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ORIGINAL PAPER

Catalytic performance and thermostability of chloroperoxidase in reverse micelle: achievement of a catalytically favorable enzyme conformation

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Abstract The catalytic performance of chloroperoxidase (CPO) in peroxidation of 2, 2′-azinobis-(-3 ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) and oxidation of indole in a reverse micelle composed of surfactant-water-isooctane-pentanol was investigated and optimized in this work. Some positive results were obtained as follows: the peroxidation activity of CPO was enhanced 248% and 263%, while oxidation activity was enhanced 215% and 222% in cetyltrimethylammonium bromide (CTABr) reverse micelle medium and dodecyltrimethylammonium bromide (DTABr) medium, respectively. Thermostability was also greatly improved in reverse micelle: at 40°C, CPO essentially lost all its activity after 5 h incubation, while 58–76% catalytic activity was retained for both reactions in the two reverse micelle media. At 50°C, about 44–75% catalytic activity remained for both reactions in reverse micelle after 2 h compared with no observed activity in pure buffer under the same conditions. The enhancement of CPO activity was dependent mainly on the surfactant concentration and structure, organic solvent ratio ($V_{pentanol}/V_{isooctane}$), and water content in the reverse micelle. The obtained kinetic parameters showed that the catalytic turnover frequency (k_{cat}) was increased in reverse micelle. Moreover, the lower K_m and higher k_{cat}/K_m demonstrated that both the affinity and specificity of CPO to substrates were improved in reverse micelle media. Fluorescence, circular dichroism (CD) and UV–vis spectra assays indicated that a catalytically favorable conformation of enzyme was achieved in reverse micelle, including the strengthening of the protein α-helix structure, and greater exposure of the heme prosthetic group for easy access of the substrate in bulk solution. These results are promising in view of the industrial applications of this versatile biological catalyst.

Keywords Chloroperoxidase · Oxidation · Peroxidation · Reverse micelle · Surfactant · Kinetics

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Introduction

Chloroperoxidase (CPO) from *Caldariomyces fumago* is a versatile hemethiolate protein, catalyzing the chlorination of activated C–H bonds and reactions reminiscent of peroxidase, catalase, and cytochrome P450. Most importantly, CPO can catalyze a variety of synthetically useful reactions with high regio- and enantio-selectivity at the expense of hydrogen peroxide or other organic hydroperoxide without the need for cofactors. The broad activities of CPO are attributed to its unique structure. CPO possesses a cysteinic thiolate as the fifth axial ligand of heme instead of the histidine imidazole ligand found in most other peroxidases [1–3].

Though CPO has been studied for a wide range of applications from synthesis of fine chemicals to detoxification of environmental pollutants, applications of industrial interest have been hindered by the frequent formation of side products and lower aqueous solubility of organic substrates, as well as its limited stability.

Enhancing the activity and stability of enzymes remains a difficult challenge because of the multiple factors involved and lack of methods allowing the evaluation of contributions from each individual parameter. Efforts to improve the activity and stability of CPO have been the subject of many studies. For example, several authors have suggested that CPO-catalyzed reactions could be carried out in aqueous co-solvent mixtures, but lower reaction rates and selectivity were often observed [4, 5]. Although the solubility of substrates was improved by performing CPO-catalyzed biotransformation in liquid–liquid systems comprising a water immiscible organic solvent and water, the catalytic efficiency and selectivity remained low [6–8]. Moreover, the thermostability of CPO was rarely improved by such processes.

Water-in-oil microemulsions with reverse micelles provides an interesting alternative to normal organic solvents in the field of enzyme catalysis with hydrophobic substrates. Reverse micelles are thermodynamically stable aggregates of surfactants. The hydrophilic head group region of reverse micelles can host proteins such as enzymes in its nanometer sized water cores. Catalytic reactions with water-insoluble substrates can occur at the large internal water–oil interface inside the microemulsions. The activity and stability of enzymes can be controlled, mainly by adjusting the molar ratio of water to surfactant (W_0). So, in recent decades, applications of reverse micelles in the recovery and separation of proteins and in enzymatic reactions have received much attention [9, 10]. However, to the best of our knowledge, the application of reverse micelle to CPO-catalyzed biotransformation has rarely been reported, with the exception of two papers on chloroperoxidase-catalyzed halogenation of apolar compounds [11] and halogenation of 1,3-dihydroxybenzene in reverse micelles [12].

Oxidation of indole, peroxidation of 2, 2′-azinobis-(-3 ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) and thermostability of CPO was investigated in reverse micelle media in this work. The micellar system was composed of the cationic surfactants DTABr/CTABr, pentanol, isooctane and water. Pentanol was used as a co-surfactant to stabilize the micelles and regulate the polarity of the micellar interphase. In many reaction systems, sodium dioctyl sulfosuccinate (AOT) is used as the surfactant because its phase behavior is well understood. However, AOT was not suitable for this work because it was found to somewhat inhibit CPO activity.

We use indole and ABTS as model substrates because elegant studies have been carried out for these reaction systems in buffer, and therefore we can focus on how reverse micelle influences the oxidation activity and peroxidation activity of CPO. Specific activities of CPO showed a 215–263% increase in the reverse micellar solution. The enhancement of CPO activity can be tuned by the water content, surfactant concentration and organic solvent ratio. Moreover, although the improvement of various enzymes in reverse micelle has been studied extensively, the mechanism involved has rarely been mentioned. In this work, fluorescence, circular dichroism (CD) and a UV–vis spectra assay were employed, showing that a catalytically favorable conformation of CPO was achieved in reverse micelle. Furthermore, an improvement of both the affinity and specificity of CPO for substrates in reverse micelle were confirmed by the kinetic parameters obtained.

Materials and methods

Materials

Chloroperoxidase was isolated from the growth medium of *Caldariomyces fumago* according to the method established by Morris and Hager [13] with minor modifications, using acetone rather than ethanol in the solvent fractionation step. The enzyme solution was concentrated to 12.6 mg/mL CPO with R_z 1.21 (R_z = purity standard = A_{398}/A_{280} = 1.44 for pure enzyme) and an activity of 5,600 U/mL based on the standard MCD assay [14].

ABTS was purchased from Aldrich (Milwaukee, WI). Indole, sodium citrate, potassium hydrogen phosphate, potassium dihydrogen phosphate, hydrogen peroxide (30% in aqueous solution), isooctane, pentanol and cationic surfactants CTABr and DTABr were from Xi'an Chemical Co. (Xi'an, PR China). All chemicals were of analytical grade unless otherwise indicated. Moreover, CTABr and DTABr were recrystallized three times by methanol/ether in order to minimize surface tension plots.

Methods

Preparation of reverse micelles

The required amount of cationic surfactants (0.025 mol CTABr and 0.03 mol DTABr) was dispersed in 500 mL isooctane-pentanol mixed solution ($V_{pentanol}/V_{isooctane}$ = 1:4) previously processed by anhydrous sodium sulfate using standard methods. Then, aqueous buffer (phosphate pH 5 or citrate pH 3) was added to obtain the desired W_0 ([g (water)]/[g (surfactant)]. The whole suspension was stirred magnetically continuously until a macroscopically homogeneous solution was formed. The aqueous stock solution of CPO was injected into the above solution using a so-called injection method to ensure high enzyme activity.

Chloroperoxidase activity assay

CPO peroxidation activity was determined using ABTS as model substrate by monitoring the formation of $ABTS^{+}$ at 415 nm ($\varepsilon_{415} = 3.6 \times 10^{-4}\ M^{-1}cm^{-1}$) [15]. All measurements were carried out at 25°C in 1 mL 0.1 M citrate buffer (pH 3) containing 5 mM ABTS, 3 mM H_2O_2, and 0.04 μM CPO.

CPO oxidation activity was measured using indole as substrate according to the absorbance decrease of indole at 270 nm [16]. It was tested at 25°C in 1 mL 0.1 M phosphate buffer (pH 5) containing 2 mM indole, 3 mM H_2O_2, and 0.04 μM CPO.

The above activities were evaluated by the specific initial reaction rate v, and calculated from the slope of changes in absorbance versus time. All sets of experiments were reproduced several times under identical operating conditions in order to ensure the data accuracy, and each point was obtained at least three times with a discrepancy below 5%.

Fluorescence measurements

A solution of 2 μM CPO was prepared in pure 0.05 M phosphate buffer or in reverse micelle (pH 5.5). The samples were excited at 287 nm, and fluorescence spectra were registered from 300 to 400 nm.

CD spectra

A solution of 0.8 μM CPO was prepared in pure 0.05 M phosphate buffer or in reverse micelle (pH 5.5) for CD assay.

Evaluation of CPO thermostability

Enzyme solutions (0.6 mM) were prepared either in pure buffer or in the reverse micellar media, and incubated at 40, 50 or 60°C, respectively. Equal aliquots were withdrawn periodically and mixed with substrate solutions at a given substrates, H_2O_2 and enzyme concentration. The thermostability of CPO was evaluated in terms of residual activity.

Determination of kinetic parameters

Kinetic assay for indole oxidation and ABTS peroxidation was carried out over the substrate concentration range of 0–200 mM. The kinetic parameters, K_m and V_{max} were determined by measuring initial rates of enzymatic reaction. Michaelis–Menten kinetic characteristics were found in all cases. K_m and V_{max} values in pure buffer and in reverse micelle were obtained by linear regression analysis of double reciprocal Lineweaver–Burk plots. The results were within experimental error.

Results and discussion

Influence of surfactant

Enhancement of CPO activity depended strongly on the surfactant concentration. A bell-shaped behavior was observed (Fig. 1), where the relative activity was expressed as the ratio of the initial reaction rate in reverse micelle to that in pure buffer. At low concentration, relative activity increased rapidly with increasing of surfactant

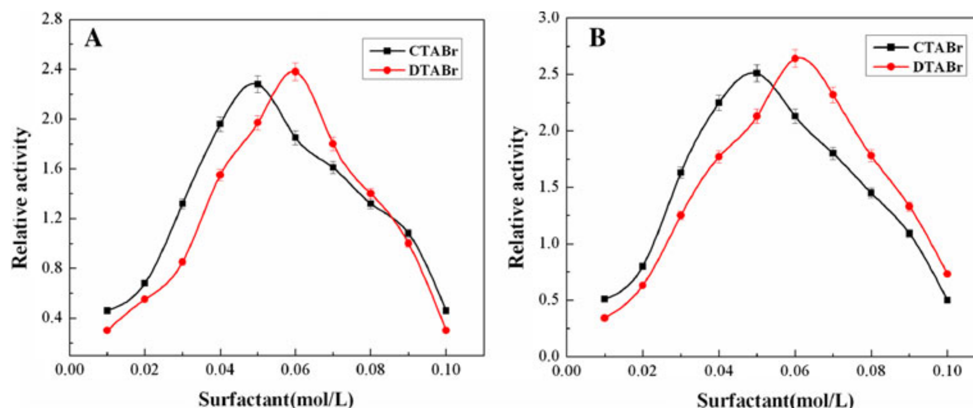

Fig. 1 Effect of surfactant concentration on the initial rate of indole oxidation and ABTS peroxidation catalyzed by chloroperoxidase (CPO) in cetyltrimethylammonium bromide (CTABr) and dodecyltrimethylammonium bromide (DTABr) reverse micelle systems at W_0 18% and W_0 21%, respectively. Reaction conditions: **a** 0.1 M phosphate buffer pH 5, 2 mM indole, 3 mM H_2O_2, and 0.04 μM CPO. **b** 0.1 M citrate buffer pH 3, 5 mM 2, 2′-azinobis-(-3 ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS), 3 mM H_2O_2, and 0.04 μM CPO. *Filled squares* CTABr (50 mM), *filled circles* DTABr (60 mM)

concentration; however, it declined at higher surfactant concentration range. The optimal peroxidation activity and oxidation activity values were 2.48 and 2.15 times higher in CTABr reverse micelle at W_0 18% than in pure buffer, and 2.63 and 2.22 times higher in DTABr reverse micelle at W_0 21%. DTABr showed a more positive effect on the catalytic performance of CPO.

The improvement of CPO activity in reverse micelle could be attributed to the interfacial activation between the water and oil, and to the emulsification of hydrophobic substrates. The size of the interfacial area usually controls the reaction rate. Thus, the surfactant mass fraction in microemulsion, which defines the interfacial area size, plays an important role, as can be seen in Fig. 1. The characteristics of the interfacial film are closely related to the length of the hydrophobic alkyl of the surfactant. Surfactants with longer alkyl chains would form a thicker interfacial film, which is not advantageous for enzyme contact with the substrate through this film. Moreover, the alkyl chain stretching out from the interface can prevent the enzyme from entering the interfacial region. In such cases, a surfactant with a shorter alkyl group (e.g., DTABr) is more effective.

The decrease in CPO catalytic activity observed at high concentrations of surfactants was probably caused by the increase of microinterface net charge, resulting in weakly electrostatic interactions of the surfactant with CPO. Moreover, CPO bears carbohydrate chains attached to its surface, which would also cause an interaction of the enzyme with the micellar matrix. Both these facts would decrease the activity of CPO as well as the flexibility of the interfacial film in reverse micelle with concentrated surfactants.

Influence of water content

Reverse micelles usually have a low aggregation number of surfactant molecules; however, this aggregation number can be greatly increased upon the addition of water, which is solubilized between the polar head groups of the surfactant, forming a so-called water pool capable of hosting proteins [17]. The results in this work showed that the enhancement of CPO activities also depended strongly on water content, expressed as W_0 ([g (water)]/[g (surfactant)] in reverse micelle, where the amount of aqueous phase was between 0.5% and 30%, and a constant $V_{pentanol}/V_{isooctane}$ value was maintained. As can be seen from Fig. 2a, CPO peroxidation activity increased with increasing W_0 at low water content range. However, a sharp decline in activity was observed when the water content was higher than 18% in CTABr reverse micelle media (20% in DTABr media).

CPO is a highly hydrophilic enzyme. The increase in CPO activity observed at low W_0 can be illustrated by the water-shell-mode, which would apply only to hydrophilic enzymes. Two aqueous regions exist within a reverse micelle. One was located in the inner part of the reverse micelle and had the same physical properties as bulk water. The other was attached to the polar head groups of the surfactant (named structured water), and differed strongly from bulk water in its physical properties, with as a lowered melting point and a lowered dielectric constant. If CPO was forced into the structured layer of water by reducing the size of the reverse micelles, the activity of CPO would decrease dramatically. So, CPO activity increased with increasing W_0.

At even higher water concentrations, the obtained hyperactivity of CPO in the reverse micelle again decreased. In this composition range, an increasing viscosity was also observed, which indicated the beginning of phase separation into a surfactant-rich aqueous phase and a water/oil (w/o)-microemulsion. Therefore, phase separation began to affect enzyme activity because the substrate was unequally distributed between the w/o-microemulsion and the aqueous phase, and access of the hydrophobic substrates to the enzyme was suppressed.

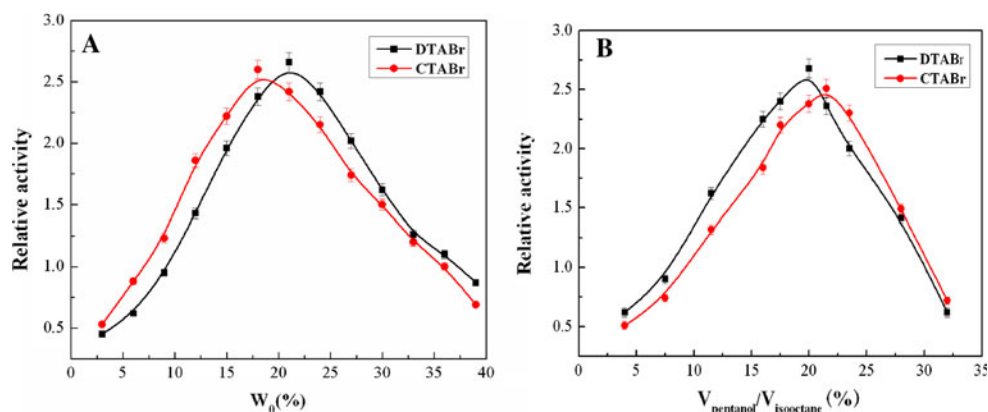

Fig. 2 Effect of **a** water content (W_0), and **b** organic solvent ratio ($V_{pentanol}/V_{isooctane}$) on peroxidation activity in reversed micelle media. Reaction conditions: 0.1 M citrate buffer pH 3, 5 mM ABTS, 3 mM H_2O_2, and 0.04 μM CPO. *Filled squares* CTABr (50 mM), *filled circles* DTABr (60 mM)

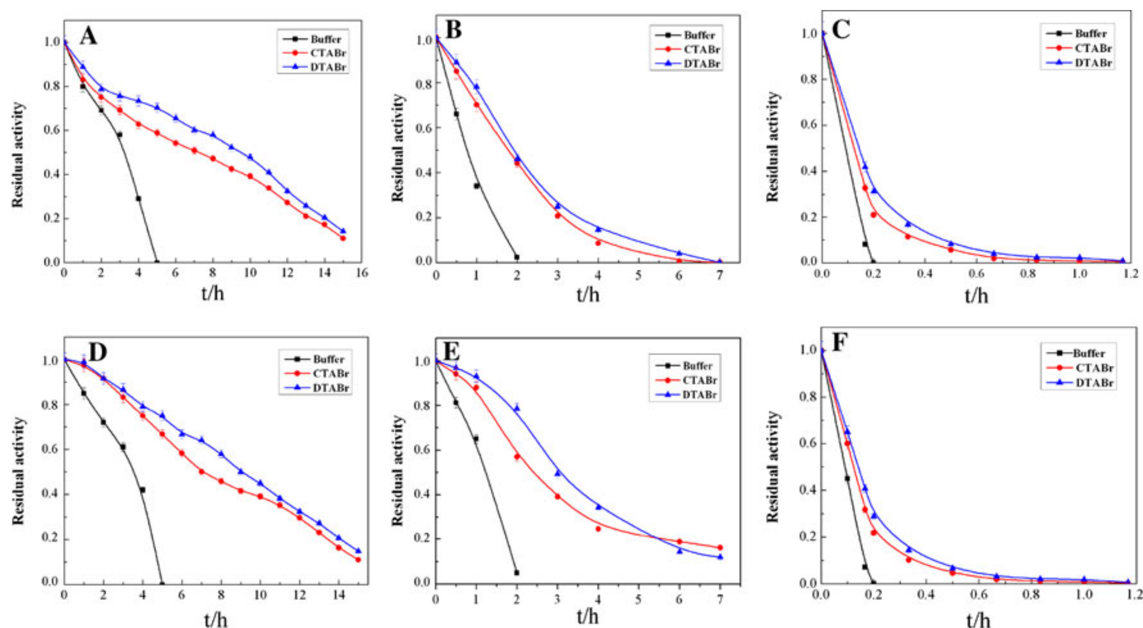

Fig. 3 Thermostability of CPO in reverse micelle. **a–c** Residual peroxidation activity of CPO at 40°C, 50°C and 60°C; **d–f** residual oxidation activity of CPO at 40°C, 50°C and 60°C. *Filled squares* Buffer, *filled circles* CTABr, *filled triangles* DTABr. Reaction conditions (**a–c**): 0.1 M citrate buffer pH 3, 5 mM ABTS, 3 mM H_2O_2, and 0.04 μM CPO for peroxidation activity measurements; **d–f**: 0.1 M phosphate buffer pH 5, 2 mM indole, 3 mM H_2O_2, and 0.04 μM CPO. CTABr media: W_0 18%, $V_{pentanol}/V_{isooctane}$ 22%, CTABr 50 mM; DTABr media: W_0 21%, $V_{pentanol}/V_{isooctane}$ 20%, DTABr 60 mM

Influence of organic solvent ratio

Reverse micelles provide an interesting alternative to normal organic solvents in enzyme catalysis with hydrophobic substrates. Catalytic reactions with water-insoluble substrates can occur at the large internal water–oil interface inside micelles. In this work, pentanol was introduced into the reverse micelles as a co-surfactant to stabilize the micelles and regulate the polarity of the water–oil interface. Figure 2b shows that the optimal activity of CPO was obtained at an organic solvent ratio ($V_{pentanol}/V_{isooctane}$) of 22% in CTABr and 20% in DTABr reverse micelles.

Thermostability of CPO in reverse micelle

Enzyme thermostability is one of the most important criteria for their industrial application. We investigated the stability of CPO at 40°C, 50°C and 60°C in reverse micelle, and compared it with CPO stability in pure buffer. Figure 3 shows that CPO was more stable at elevated temperatures in reverse micelle, and an improved catalytic performance was achieved. At 40°C, CPO essentially lost all activity after 5 h incubation, while 58–76% catalytic activity was retained for both reactions in the two reverse micelle media. At 50°C, about 44–75% activity remained for both reactions in reverse micelle after 2 h compared with no observed activity in pure buffer under the same conditions. However, the protective effect on CPO of reverse micelle was unsatisfactory at higher temperatures; for example, CPO was inactivated rapidly at 60°C—within 12 min in pure buffer and within 50 min in reverse micelle media. The deactivation constant (K_d) and half-life ($t_{1/2}$) for an exponential decay of activity [$E = E^o \exp(-K_d t)$] for buffer and reverse micelle experiments at each temperature was calculated based on the inactivation kinetic characteristics, and is presented in Table 1.

These results indicated that CPO could be protected efficiently in reverse micelle at high temperature range. This could be because emulsification of the enzyme in reverse micelle prevented it from conformational distortion or protein denaturation imposed by heat.

Fluorescence and CD assay to detect structural and conformation changes in CPO in reverse micelle

The dominant fluorophore in proteins is the tryptophan (Trp) residue. Fluorescence yield is very strongly correlated with local environment, which makes fluorescence properties an excellent probe for conformational changes in a protein's interior. The fluorescent spectrum of CPO showed that the intensity of maximum emission around 337 nm increase markedly in reverse micelle (especially in DTABr media); however, no red-shift of this emission peak accompanied this increase (Fig. 4), indicating that there

Table 1 The half-life ($t_{1/2}$) and deactivation constant (K_d) for exponential decay of activity in buffer and in reverse micelle at 40°C, 50°C and 60°C

Medium	Peroxidation						Oxidation					
	$t_{1/2}$ (h)			K_d (h^{-1})			$t_{1/2}$ (h)			K_d (h^{-1})		
	40°C	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C	40°C	50°C	60°C
Buffer	3.20	0.75	0.10	0.2166	0.9242	6.9314	3.50	1.25	0.10	0.1980	0.5545	6.9314
CTABr	7.00	1.58	0.13	0.0990	0.4387	5.3319	7.00	2.50	0.13	0.0990	0.2773	5.3319
DTABr	9.30	2.00	0.15	0.0745	0.3466	4.6209	9.00	3.10	0.15	0.0770	0.2236	4.6209

CTABr Cetyltrimethylammonium bromide, *DTABr* dodecyltrimethylammonium bromide

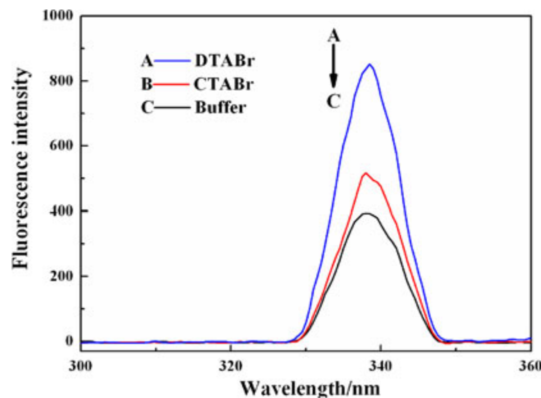

Fig. 4 Fluorescent spectrum of CPO in DTABr or CTABr reverse micelle, or pure buffer. Composition of reverse micelle: W_0 18%, $V_{pentanol}/V_{isooctane}$ 22%, CTABr 50 mM; W_0 21%, $V_{pentanol}/V_{isooctane}$ 20%, DTABr 60 mM

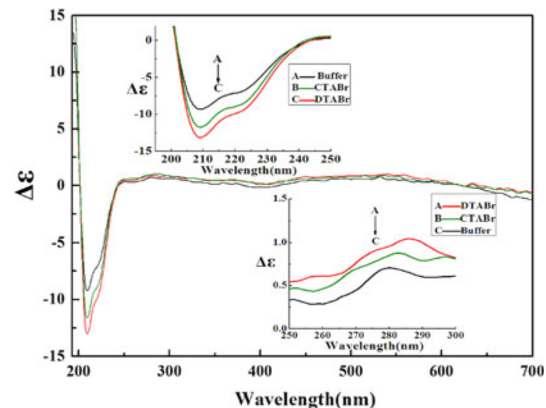

Fig. 5 Circular dichroism (CD) spectrum of CPO in DTABr, CTABr reverse micelle or pure buffer. Composition of reverse micelle: W_0 18%, $V_{pentanol}/V_{isooctane}$ 22%, CTABr 50 mM; W_0 21%, $V_{pentanol}/V_{isooctane}$ 20%, DTABr 60 mM

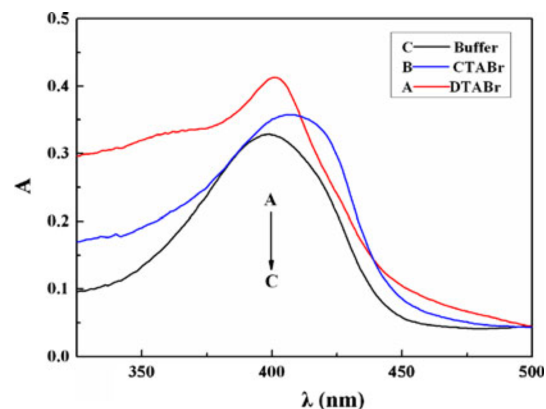

Fig. 6 UV–vis spectrum of CPO in DTABr, CTABr reverse micelle or pure buffer at pH 5.5. Composition of reverse micelle: W_0 18%, $V_{pentanol}/V_{isooctane}$ 22%, CTABr 50 mM; W_0 21%, $V_{pentanol}/V_{isooctane}$ 20%, DTABr 60 mM

was no obvious unfolding of CPO in reverse micelle. The increase in fluorescence yield could be attributed to the energy transfer from Tyr to Trp, resulting in a fluorescence quenching of Tyr with a corresponding increase in Trp fluorescence. This implies that the α-helix structure of CPO was strengthened in reverse micelle, thus decreasing the distance between Tyr and Trp. This conclusion was also confirmed by CD assay.

CD spectroscopy, which was employed to investigate the structure and conformation of CPO in this process, showed that in the "far-UV" spectral region (190–250 nm), there was a large negative absorption peak around 208 nm, which demonstrated that CPO is a typical α-helix protein (Fig. 5). This negative absorption increased in reverse micelle, which implied the α-helix structure of CPO was strengthened. Moreover, the increase of this negative absorption was more obvious in DTABr than that in CTABr reverse micelle, indicating DTABr reverse micelle media was more effective for CPO activity enhancement. This observation was consistent with the above conclusion from the fluorescence assay. In the "near-UV" spectral region (250–350 nm), the increase in CD signal around 276–288 nm in reverse micelle revealed the corresponding change of tertiary structure.

UV–vis spectrum assay of the heme micro-environment of CPO in reverse micelle

The available crystal structures of CPO show that the heme edge is not accessible directly. However, there is a small

Table 2 Kinetic parameters of 2, 2′-azinobis-(-3 ethylbenzothiazoline-6-sulfononic acid) diammonium salt (ABTS) peroxidation and indole oxidation catalyzed by chloroperoxidase (CPO) in reverse micelle systems

Medium	Peroxidation			Oxidation		
	K_m (10^{-2}/mol L^{-1})	k_{cat} (10^5/S^{-1})	k_{cat}/K_m (10^6/mol^{-1} S^{-1} L)	K_m (10^{-2}/mol L^{-1})	k_{cat} (10^5/S^{-1})	k_{cat}/K_m (10^6/mol^{-1} S^{-1} L)
Buffer	5.69 ± 0.07	3.43 ± 0.03	6.03 ± 0.06	7.21 ± 0.02	1.46 ± 0.02	2.02 ± 0.07
CTABr	5.09 ± 0.05	4.48 ± 0.06	8.80 ± 0.08	6.25 ± 0.07	1.98 ± 0.06	3.17 ± 0.11
DTABr	4.86 ± 0.03	4.83 ± 0.05	9.92 ± 0.12	5.77 ± 0.18	2.46 ± 0.07	4.26 ± 0.07

The substrate concentration varied over the 0.00–200.00 mM range

opening above the heme that could allow access of substrate to the active center. But the size and structure of substrates would be restricted by this channel, which connects the surface to the heme moiety. UV–vis spectrum of CPO in reverse micelle showed that the absorption of the CPO Soret band at $\lambda_{max} = 398$ nm was increased compared to that in pure buffer (Fig. 6), which could suggest that the heme ring is more exposed in reverse micelle, and thus it is easier for CPO to bind substrates in such environments. But in CTABr reverse micelle, a small red-shift to λ_{max} from 398 to 408 nm was observed, which may be induced by the binding of solvent H_2O to the heme iron at the sixth coordinating position because of the exposure of heme to bulk solution.

Kinetic assays

The measured kinetic parameters are given in Table 2. Compared with that in aqueous buffer solutions, the catalytic turnover frequency (k_{cat}) increased while the Michaelis constant (K_m) decreased for both the peroxidation and oxidation reactions in reverse micelle, indicating that the affinity and selectivity of CPO for the substrate was improved in these systems.

The observed kinetic behavior may be attributed to the fact that forcing the enzyme into the water core in reverse micelle may induce a favorable conformational change for both the catalytic activity and stability of CPO.

Conclusion

The results in this work showed that both the activity and the thermostability of CPO were improved in DTABr/CTABr–isooctane–water–pentanol reverse micelle media. This solution improved the solubility of the hydrophobic substrate and provided a more homogeneous environment for the enzyme-catalyzed reaction.

Surfactant structure and concentrations play an important role in the process. At lower surfactant concentration range, catalytic activities of CPO increased dramatically with an increase in surfactant concentration, while a decrease was observed at higher surfactant concentrations. Surfactants with shorter alkyl chains (e.g., DTABr) were more effective at improving CPO activity.

The enzyme activity had a bell-shaped dependence on water content in reverse micelle. The diameter of the water pool in reverse micelle matched the enzyme molecule size at optimum $W_{0.}$ A co-surfactant was required to stabilize the cationic micelles and regulate the polarity of the micelle inter-phase.

A catalytically favorable conformation of CPO was achieved in reverse micelle, including strengthening of the α-helix structure and greater exposure of heme for easy access of the substrate, resulting in an increase in the enhancement of catalytic turnover frequency (k_{cat}) and an improvement in the affinity and selectivity of CPO for its substrate.

These findings demonstrate that CPO catalytic behavior can be improved using a solvent engineering approach.

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References

1. Sundaramoorthy M, Terner J, Poulos TL (1995) The crystal structure of chloroperoxidase: a heme peroxidase-cytochrome P450 functional hybrid. Structure 3:1367–1377
2. Adam W, Lazarus M, Saha-Möller CR, Weichold O, Hoch U, Häring D, Schreier P (1999) Biotransformations with peroxidases. Adv Biochem Eng Biotechnol 63:73–108
3. Dembitsky VM (2003) Oxidation, epoxidation and sulfoxidation reactions catalysed by haloperoxidases. Tetrahedron 59: 4701–4720
4. Chiappe C, Neri L, Pieraccini D (2006) Application of hydrophilic ionic liquids as co-solvents in chloroperoxidase catalyzed oxidations. Tetrahedron Lett 47:5089–5093
5. Narayanan R, Zhu G, Wang P (2007) Stabilization of interface-binding chloroperoxidase for interfacial biotransformation. J Biotechnol 128:86–92
6. Zhu G, Wang P (2005) Novel interface-binding chloroperoxidase for interfacial epoxidation of styrene. J Biotechnol 117:195–202
7. Narayanan R, Zhu G, Wang P (2007) Stabilization of interface-binding chloroperoxidase for interfacial biotransformation. J Biotechnol 128:86–92

8. Tzialla AA, Kalogeris E, Gournis D, Sanakis Y, Stamatis H (2008) Enhanced catalytic performance and stability of chloroperoxidase from *Caldariomyces fumago* in surfactant free ternary water-organic solvent systems. J Mol Catal B Enzym 51:24–35
9. Martinek K, Levashov AV, Klyachko N, Khmelnitski YL, Berezin IV (1986) Micellar enzymology. Eur J Biochem 155: 453–468
10. Shome A, Roy S, Das PK (2007) Nonionic surfactants: a key to enhance the enzyme activity at cationic reverse micellar interface. Langmuir 23:4130–4136
11. Franssen MCR, Weijnen JGJ, Vincken JP, Lane C, Van der Plas HC (1988) Chloroproxidase-catalysed halogenation of apolar compounds using reversed micelles. Biocatalysis 1:205–216
12. Chen J, Zhang J, Han B, Li J, Li Z, Feng X (2006) Effect of compressed CO_2 on the chloroperoxidase catalyzed halogenation of 1, 3-dihydroxybenzene in reverse micelles. Phys Chem Chem Phys 8:877–881
13. Morris DR, Hager LP (1966) Chloroperoxidase I. Isolation and properties of the crystalline glycoprotein. J Biol Chem 241:1763–1768
14. Hager LP, Morris DR, Brown FS, Eberwein H (1966) Chloroperoxidase II. Utilization of halogen anions. J Biol Chem 241: 1769–1777
15. Reszka KJ, Britigan BE (2007) Doxorubicin inhibits oxidation of ABTS by a lactoperoxidase/H_2O_2 system by reacting with ABTS-derived radical. Arch Biochem Biophys 466:164–171
16. Zhi L, Jiang Y, Wang Y, Hu M, Li S, Ma Y (2007) Effects of additives on the thermostability of chloroperoxidase. Biotechnol Prog 23:729–733
17. Orlich B, Schomäcker R (2002) Enzyme catalysis in reverse micelles. Adv Biochem Eng Biotechol 75:185–208